α -Endosulfine regulates amyloid β 42 via the modulation of neprilysin activity

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Abstract

The neuropeptide somatostatin (SST) regulates amyloid β peptide (A β) catabolism by enhancing neprilysin (NEP)-catalyzed proteolytic degradation. However, the mechanism by which SST regulates NEP activity remains unclear. Here we report the identification by differential proteomics of α -endosulfine (ENSA), an endogenous ligand of the ATP-sensitive potassium (K_{ATP}) channel, as a negative regulator of NEP activity downstream of SST signaling. Genetic deficiency of ENSA resulted in enhanced NEP activity and decreased A β deposition in the brains of wild-type and Alzheimer's disease (AD) model mice. Pharmacological intervention to increase the probability of K_{ATP} channel opening reduced A β deposition in AD model mice. Our findings provide new insights into possible mechanisms to prevent AD.

Introduction

Accumulation of amyloid β peptide (A β) in the brain is a pathological hallmark of Alzheimer's disease (AD). To this end, the identification of pathogenic mutations in the *APP*, *PSEN1* and *PSEN2* genes supports the amyloid cascade hypothesis underlying the etiology of AD¹, and verify that these mutations cause early-onset AD due to the abnormal production and accumulation of toxic A β species such as A β_{42} and A $\beta_{43}^{2,3}$. In contrast, the exact causes of A β deposition in sporadic AD cases remain unclear, although some genetic risk factors related to A β metabolism have been identified⁴.

Neprilysin (NEP; neutral endopeptidase 24.11) is a major physiological A β -degrading enzyme^{5,6}, the expression of which in the brain declines with aging and in early stages of AD progression^{7,8}. Identification of the mechanism(s) that regulate NEP activity should contribute to the development of ways to prevent AD. We previously showed that somatostatin (SST), a neuropeptide known as a somatotropin-release inhibiting hormone⁹, regulates A β_{42} levels in the brain via the upregulation of NEP activity¹⁰. In addition, we discovered that, of the five SST receptor (SSTR1-5) subtypes, SSTR1 and SSTR4 redundantly regulate NEP activity and

modulate $A\beta_{42}$ levels in the brain^{11,12}. In the present study, we address how NEP activity is regulated in the signaling cascade downstream of SST.

Using in vitro proteomics, we identified ENSA, an endogenous ligand of the ATP-sensitive potassium (K_{ATP}) channel ^{13,14}, as a negative regulator of NEP downstream of SST signaling. To analyze the role of ENSA in vivo, we generated ENSA-deficient mice using genome-editing tools. ENSA deficiency induced NEP activation and reduced Aβ level in the brains of wild-type (WT) and App knock-in mice, which develop amyloid plaques without overexpression of APP gene containing pathogenic mutations¹⁵. We also determined that ENSA is a substrate for NEP both in vitro and in vivo. Consistently, the expression of ENSA was higher in App knock-in mice and AD patients than in controls. We also show that diazoxide (Dz), a K_{ATP} channel agonist used for the treatment of hypoglycemia and other conditions^{16,17}, improved amyloid pathology and memory impairment in App knock-in mice. Our results establish a molecular link between the KATP channel and NEP activation and provide new insights into the development of alternative strategies to prevent AD.

Results

Identification of ENSA as a regulator of NEP activity in vitro. We previously developed a method for measuring NEP activity in primary neurons,¹⁰ and subsequently developed a coculture system composed of cortical/hippocampal and basal ganglia neurons in a ratio of 9:1, which contain both SSTR1 and SSTR4.11 Treatment of mixed culture of neurons from different brain regions of E16-18 C57BL/6Ncr mice, but not of neurons from the individual brain regions, with SST or TT232, a selective agonists for SSTR1 and SSTR4, respectively, elevated NEP activity (Fig. 1a-c). Co-cultured neurons derived from SSTR1 and SSTR4 double knockout (Sst₁/Sst₄ dKO) mice failed to exhibit the SST-induced NEP upregulation (Extended Data Fig. 1ac). We next examined if neurons from cortical/hippocampal and/or basal ganglia origin generate a secretory factor that activates NEP in the mixed culture neurons. Cultured cortical/hippocampal and basal ganglia neurons were treated separately with SST, and the collected conditioned media added to co-cultured neurons (Fig. 1d). We collected media from SST-treated WT cortical/hippocampal neurons (Media A) and from basal ganglia neurons (Media B), and found that only Media A significantly elevated the NEP activity of co-cultured neurons (Fig. 1e-f). Media A also elevated NEP activity in co-cultured neurons derived from Sst₁/Sst₄ dKO mice

(Extended Data Fig. 1d,e), indicating that the NEP-stimulating element in question is secreted by cortical/hippocampal neurons and not basal ganglia neurons, and that NEP activity is upregulated in a manner independent of the SST-SSTR pathway.

A centrifugal filter was used to concentrate NEP activity modulators to the 10-30 kDa molecular weight range (Extended Data Fig. 1f-j), and this fraction was then subjected to LC-MS/MS analysis to identify candidate mediators. Initially, we performed a qualitative comparison between proteins identified in the conditioned media from wild-type primary neurons treated with or without SST and TT232. We also used conditioned media from Sst₁/Sst₄ dKO mice in the LC-MS/MS analysis as a negative control. We then searched for proteins absent or present only in the media of the SST- and TT232-treated WT mixed neurons, but not in the media of Sst₁/Sst₄ dKO neurons. In this way, we identified three candidate proteins: (1) ENSA, (2) neuron-specific protein family member 1 (NSG-1) and (3) nuclear ubiquitous casein and cyclin-dependent substrate 1 (NUCKS-1) (Table 1). To determine which of the candidates is involved in the regulation of NEP activity, we analyzed the effects of corresponding recombinant proteins on NEP activity in cocultured neurons. Only the recombinant ENSA decreased NEP activity in co-cultured neurons from WT and Sst1/Sst4 dKO mice in a dose-dependent manner (Fig. 1g-i and Extended Data Fig. 1k). Consistent with this, treatment of the co-cultured neurons from WT mice, but not from ENSA-deficient mice, with an antibody that specifically neutralizes ENSA (GTX101493) significantly increased NEP activity at a dose of 100 ng/ml (Extended Data Fig. 11,m).

We next analyzed ENSA levels in the brains of *Sst*₁/*Sst*₄ dKO mice and SST knock out (*Srif* KO) mice and found that ENSA levels were significantly elevated in the cortex and hippocampus of both mouse strains (Fig. 1j-l). In addition, immunohistochemical analyses indicated that ENSA-positive signals were heightened in the cortical and hippocampal CA1 and CA3 regions in these animals (Fig. 1m-p). Taken together, these results suggest that ENSA functions both *in vitro* and *in vivo* as a negative NEP regulator downstream of SST signaling.

Activation of NEP by genetic deficiency of ENSA *in vivo*. ENSA, an endogenous ligand of sulfonylurea receptor 1, regulates the secretion of insulin from pancreatic β cells and is highly expressed in brain and skeletal muscle and at lower levels in the pancreas^{13,14}. Although we found that ENSA is a novel negative regulator of NEP *in vitro*, the function of ENSA *in vivo* is largely unknown. We therefore generated ENSA knock out (*Ensa* KO) mice using CRISPR/Cas9 technology. Dual adjacent single-guide RNAs (sgRNAs) were designed that targeted exon 1 of

the Ensa gene including the initiation codons (Extended Data Fig. 2a). This strategy facilitates CRISPR-mediated genome targeting¹⁸. We injected sgRNA1-Ensa-Exon1 (30 ng/ml) and sgRNA2-Ensa-Exon1 (30 ng/ml) together with Streptococcus pyogenes Cas9 (SpCas9) mRNA (60 ng/ml) into WT mouse zygotes. Sanger sequencing analysis and PCR-based genotyping indicated the deletion of exon 1, including the initiation codons (Extended Data Fig. 2b,c). Expression of ENSA in homozygous F2 mutant lines, generated by crossbreeding the heterozygous F1 mutant lines, was fully deleted (Extended Data Fig. 2d,e). To assess the offtarget effects of CRISPR/Cas9 in the founder, we searched for potential off-target sites using COSMID, with 55 candidate sites being identified (Supplemental Table 1)¹⁹. Of note, there was no off-target mutation on chromosome 3, which contains the *Ensa* gene. PCR-based genotyping and Sanger sequencing analyses for each candidate site revealed that founder had an off-target mutation in an intergenic region of chromosome 2 which was removed by backcrossing the mutant mice with WT mice (Extended Data Fig. 2f, g).

NEP efficiently degrades $A\beta_{42}$ in the presynaptic region rather than inside secretary vesicles¹⁰. To determine whether a deficiency of ENSA affects the localization of NEP, we used immunohistochemistry to analyze the expression of NEP and vesicular GABA transporter

(VGAT; a presynaptic marker) in the brains of *Ensa* KO mice. We found that NEP signals in the outer molecular layer of the dentate gyrus (OMo) were significantly increased (Fig. 2a,b), and that colocalization of NEP and VGAT these mice was increased in both the lacunosum molecular layer (LM) and OMo (Fig. 2a,c). Next, we measured NEP activity in hippocampal membrane fractions from Ensa KO mice and found that a deficiency of ENSA paralleled that of a significantly increased NEP activity (Fig. 2d). We then quantified $A\beta_{40}$ and $A\beta_{42}$ levels in the hippocampi of Ensa KO mice by enzyme-linked immunosorbent assay (ELISA) and found that A β_{42} levels were significantly reduced compared to those of control mice (Fig. 2f), with A β_{40} levels remaining relatively stable (Fig. 2e). This reduction of $A\beta_{42}$ was reproduced in another line of Ensa KO mice (Ensa KO #2) that was generated by CRISPR/Cas9 with different sgRNAs (Extended Data Fig. 3). These results are in agreement with the effect of somatostatin deficiency¹⁰. AB42 levels in ENSA and NEP double knock out (Ensa/Mme dKO) mice did not differ from those of single *Mme* KO mice (Fig. 2g), indicating that NEP mediated the reduction of A β_{42} in the hippocampi of Ensa KO mice.

We next investigated whether the deficiency of ENSA affected the processing of $A\beta$ production or expression of other $A\beta$ -degrading enzymes. We performed Western blot analysis of full-length APP, its C-terminal fragments generated by α-secretase (CTF-α) and β-secretase (CTF-β), insulindegrading enzyme (IDE), and endothelin converting enzyme 1 (ECE-1). No significant differences were observed in the expression levels of these proteins and fragments (Extended Data Fig. 2h). Mitogen-activated Protein Kinase/Extracellular Signal-regulated Kinase (ERK1/2) and protein phosphatase 1 (PP1) regulates NEP's cell surface localization thorough modulation of phosphorylation status in intracellular domain of NEP²⁰. The phosphorylation statuses of ERK1/2 at the threonine 202 and tyrosine 204 and PP1 at the threonine 320 residues, which indicate their activity condition respectively^{21,22}, however, remained unchanged in *Ensa* KO mice (Extended Data Fig. 4a-c).

To examine the effect of ENSA deficiency on A β pathology, we next crossbred *Ensa* KO mice with $App^{NL-F/NL-F}$ Knock-in (App^{NL-F}) mice. App^{NL-F} mice harbor two familial AD-causing mutations (Swedish (KM670/671NL) and Beyreuther/Iberian (I716F)) in the endogenous Appgene as well as humanized A β sequences, and develop amyloid pathology in the hippocampus and cortex from around 6 months of age¹⁵. The percentage of amyloid plaque deposition in hippocampal molecular layer area was significantly reduced in $App^{NL-F}/Ensa$ KO mice, where NEP expression was elevated (Fig. 2h,i). This result was confirmed in A β ELISA experiments on

the hippocampi of these mice which showed that $A\beta_{42}$ was significantly decreased (Fig. 2j,k). We consistently found that NEP expression in the LM and OMo of $App^{NL-F}/Ensa$ KO mice was upregulated, particularly in the presynaptic region of OMo (Fig. 2l-n). Taken together, these observations suggest that ENSA is a negative regulator of NEP activity *in vivo* and that a deficiency of ENSA attenuates A β pathology by allowing NEP activity to be upregulated.

NEP activity was also measured in cardiac fractions from *Ensa* KO mice given that LCZ696, a dual-acting angiotensin-receptor-neprilysin inhibitor drug, has been approved and is being used to treat heart failure.^{23,24} NEP activity was unaltered in the heart and kidney of *Ensa* KO mice compared to WT controls (Extended Data Fig. 5a,b). Indeed, NEP expression in the kidney was much higher than that in the heart, which is consistent with a previous report stating that the kidney expresses the highest level of NEP among all the mammalian organs²⁵.

Feedback mechanism regulating NEP activity. Previously, several substrates for NEP such as enkephalin, neuropeptide Y and A β were identified²⁶⁻²⁹. SST is an endogenous upregulator of NEP and is also degraded by NEP in a substrate-dependent feedback manner¹⁰. We hypothesized that NEP might also directly degrade ENSA in a similar feedback manner. Co-incubation of

recombinant ENSA with NEP resulted in a remarkable decrease in ENSA levels (Fig. 3a). Several NEP inhibitors such as thiorphan, phosphoramidon and EDTA attenuated this effect, indicating that NEP degrades ENSA *in vitro* (Fig. 3a). In contrast, NSG-1 and NUCKS-1 were not cleaved by NEP (Extended Data Fig. 6a). To identify the NEP-mediated cleavage sites in ENSA, we performed MALDI-TOF analysis after incubation of recombinant ENSA with NEP. Several ENSA fragments were detected in the NEP-treated sample, but not in a sample treated in the presence of thiorphan (Fig. 3b, c and Extended Data Fig. 7). We determined the amino acid sequences of these fragments by LC-MS/MS analysis (Supplemental Table 4), and found that NEP partially cleaved ENSA on the amino-terminal side of hydrophobic amino acids in a manner similar to that of other NEP substrates, including Aβ (Fig. 3d).

ENSA levels in the brains of *Mme* KO mice were subsequently examined by Western blotting and we observed that ENSA was significantly increased in the hippocampi and cortices of these animals (Fig. 3e,f and Extended Data Fig. 6b,c). We then overexpressed WT and inactivated mutant NEP in the hippocampi of WT mice using the Semliki Forest virus (SFV) gene expression system³⁰. Exogenously expressed active NEP, but not the inactive mutant, significantly lowered ENSA as well as $A\beta_{42}$ in hippocampi (Fig. 3g-i Extended Data Fig. 6d). We also performed immunohistochemical analyses of ENSA and NEP and found that these two proteins co-localized in the CA3 region (Fig. 3j). These results suggest that NEP directly contributes to the degradation of ENSA *in vivo* and that NEP activity is regulated by a substrate-dependent feedback mechanism.

Elevated ENSA levels in an AD mouse model and in AD patients. To explore the involvement of ENSA in the etiology of AD, we analyzed ENSA levels in an AD mouse model and in postmortem brain of patients with AD. Western blot analyses revealed that ENSA expression was significantly increased in the cortices and hippocampi of 12- and 24-month-old App^{NL-F} mice, but not in those of 2-month-old mice which do not yet exhibit amyloid deposition (Fig. 4a-d and Extended Data Fig. 8a-h). In immunohistochemical analyses, ENSA signals in the cerebral cortices and hippocampal CA1 and CA3 regions of App^{NL-F} mice were also increased at 24 months (Fig. 4e,f). Consistent with these observations, ENSA levels were markedly increased in the cortices of patients with AD (Fig. 4i-l). In contrast, the mRNA levels of ENSA in App^{NL-F} mice did not differ from those of WT mice at the age of 12 and 24 months (Fig. 4g,h and Extended Data Fig. 8i,j), implying that the proteostasis of ENSA was perturbed in the brains of AD mice.

While WT A β_{42} inhibited the NEP-mediated degradation of ENSA in a dose-dependent manner

in vitro (Fig.4 m,n and Extended Data Fig. 8k,m), mutated A β_{42} with the Arctic mutation, that escapes proteolytic degradation by NEP³¹, failed to exert such an effect (Fig.4 m,n and Extended Data Fig. 8l,m). Consistent with this, another AD mouse model, $App^{NL-G.F/NL-G.F}$ Knock-in ($App^{NL-G.F}$), that harbors the Arctic mutation in addition to the Swedish and Beyreuther/Iberian mutations and exhibits a more aggressive A β pathology than App^{NL-F} mice, showed ENSA levels in the cortex and hippocampus at 6 months (Fig. 4o-r) that were indistinguishable from those of WT mice. ENSA levels in 24-month-old App^{NL-G-F} mice with more aggressive inflammation than App^{NL-F} mice were significantly reduced (Extended Data Fig. 8n-r). These results suggest that the elevation of ENSA levels in AD is due to a competitive inhibition between ENSA and A β_{42} of NEP-mediated degradation.

Improvement of $A\beta$ pathology and memory function by diazoxide in an AD mouse model. ENSA is known to function as a blocker of the K_{ATP} channel¹³. To investigate whether the K_{ATP} channel modulates NEP activation, we incubated mouse primary neurons with diazoxide (Dz), a K_{ATP} channel agonist, and found that this activated NEP in a dose-dependent manner (Fig. 5a). As Dz has been reported cross the blood brain barrier,^{16,17} we treated WT mice by oral administration of Dz for 1 month. This treatment significantly increased NEP activity in the anterior cortex and hippocampus (Fig. 5b) of these animals, with elevated levels of NEP expression also seen in the anterior cortex (Fig. 5c,d). In line with this, Dz significantly lowered $A\beta_{42}$ levels in the anterior cortex and hippocampus, where NEP was activated (Fig. 5e), whereas Dz treatment had no effect on $A\beta_{42}$ levels in the anterior cortex and hippocampus of *Mme* KO mice (Fig. 5f). These results suggest that Dz decreased $A\beta_{42}$ levels in a NEP-mediated manner.

We next investigated the therapeutic effect of Dz on App^{NL-F} mice by carrying out contextual fear-conditioning tests to assess memory function after 3 months of Dz treatment from the age of 15 months. Dz treatment recovered the freezing ratio of App^{NL-F} mice to a level comparable to that of WT mice (Fig. 5g). We also performed open field tests to assess the anxiety phenotype as it has been shown that anxiety may affect performance in spatial memory tasks³². Dz treatment in WT and App^{NL-F} mice did not alter the amount of time spent in the central region of the open field maze (Extended Data Fig. 9a), indicating that Dz had no effect on psychological status. In contrast, the Dz treatment improved abnormal spatial memory function in aged App^{NL-F} mice, and also decreased A β plaque deposition in the cortex, subiculum and hippocampal molecular layer (Fig. 5h,j). A β_{42} levels in the cortex and hippocampus of these animals were also reduced (Fig. 5j).

Immunohistochemical analyses indicated an increase in NEP expression in the anterior cortex of these mice (Extended Data Fig. 9b,c). In addition, co-localized signals of NEP and VGAT were also increased in the presynaptic regions of the hippocampal LM and OMo (Fig. 5k-m). Dz had no effect on behavior (Extended Data Fig. 9d,e) or A β pathology in *App^{NL-F}/Mme* KO mice (Extended Data Fig. 9f,g). Taken together, these results suggest that Dz improves A β pathology and memory impairment in *App^{NL-F}* mice by upregulating NEP activity.

Discussion

In the present study, we used *in vitro* and *in vivo* experimental paradigms to identify ENSA as a negative regulator of NEP activity. A genetic deficiency of ENSA increased NEP activity and markedly lowered $A\beta_{42}$ levels. In addition, ENSA was identified as a substrate for NEP, suggesting a potential feedback mechanism for the regulation of NEP activity. Consistently, ENSA levels were found to be increased in AD model mice and AD patients. Moreover, while ENSA functions as an endogenous blocker of the K_{ATP} channel, using Dz as an agonist of the channel prevented $A\beta$ deposition via the activation of NEP and improved memory function in AD model mice. The key findings in this study are schematized in Extended Data Fig.10.

While ENSA plays an important role in cell cycle regulation in several cell types³³⁻³⁵, its

function in the central nervous system however remains largely unknown. Our experiments suggested that ENSA is involved in the A β catabolic pathway, achieving its effects via the modulation of NEP activation. A deficiency of ENSA accelerates the translocation of NEP from intracellular secretary vesicles to the presynaptic surface, and while the exact mechanism by which this occurs is unclear, our data indicate that phosphorylation levels of ERK and PP1 in *Ensa* KO mice did not differ from those of WT mice (Extended Data Fig. 4a-c), suggesting that an alternative mechanism regulates the localization of NEP *in vivo*, which remains to be explored.

SST mRNA levels were reported to decrease in brain with aging and in $AD^{26,36,40}$. As such, ENSA, a downstream protein of SST signaling, may be related to the etiology of AD. Indeed, we showed elevation of ENSA levels in the cortices and hippocampi of 12- and 24-moth-old App^{NL-} F mice as well as in the cortices of AD patients (Fig. 4a-f,i-l and Extended Data Fig. 8e-h). Moreover, *in vitro* and *in vivo* experiments revealed that NEP degraded ENSA as a substrate, suggesting that NEP and ENSA form a negative feedback loop. This hypothesis is based on the fact that opioids and substance P, cell-specific ligands in monocytes and bone marrow cells, respectively^{41,42}, regulate NEP via a feedback mechanism. It is possible that A β and ENSA

feedback-loop and inducing a vicious cycle.

A selective agonist of the KATP channel such as Dz could serve as a beneficial approach to break this vicious cycle given that it is used as a drug for antihypertensive and hypoglycemic properties, and has the potential in the preclinical setting to improve behavioral abnormalities and Aß pathology in AD^{16,17}. A previous study showed that Dz treatment reduced the extracellular accumulation of A β in 3xTg mice which display both amyloid and tau pathology due to overexpression of mutated APP and MAPT genes on a mutant PSEN1 background^{43,44}. The mechanism by which Dz attenuated Aß plaque deposition was, however, unclear. Our findings indicate that Dz reduced amyloid deposition in App^{NL-F} mice via the regulation of NEP activity in the anterior cortex and hippocampus. This regional selectivity of NEP regulation by Dz may be dependent on the dopaminergic system in the brain. The KATP channel is highly expressed in dopaminergic neurons in the midbrain and regulates dopamine release. These neurons project to the frontal cortex and hippocampus⁴⁵⁻⁵⁰. Recently we confirmed that dopamine regulates NEP expression and/or activity in the anterior cortex and hippocampus region (N.W, N.K. & T.C.S unpublished data). To further elucidate the mechanism for the regulation of NEP activity, it will be necessary to investigate pathways downstream of ENSA. Likewise, it is important to clarify

which K_{ATP} channel subtypes are involved in the regulation of NEP activity in the brain to avoid off-target effects given that different K_{ATP} channel subtypes are expressed in vascular smooth muscle cells, cardiac muscle cells and pancreatic β -cells⁵¹. In addition to promoting NEPmediated A β degradation, K_{ATP} channel agonists may have beneficial effects in AD. Dz treatment prevents A β -induced neurotoxicity induced by oxidative stress and inflammatory damage and also shows neuroprotective effects against apoptosis *in vitro*⁵²⁻⁵⁶. Compared to A β -targeting immunotherapies, synthetic agonists for the K_{ATP} channel are less expensive and would be more acceptable in aging societies around the world. Taken together, we have demonstrated here a new preventive approach at the preclinical stage of AD based on the function of ENSA. This negative regulator of NEP and K_{ATP} channel (via which its effects are mediated) could be a new therapeutic target for lowering A β .

Materials and Methods

Animals

All animal experiments were conducted according to guidelines of the RIKEN Center for Brain Science. *Srif* KO and *Sst*⁴ KO mice were kindly provided by Ute Hochgeschwender, Oklahoma Medical Research Foundation as previously described¹¹. *Sst*¹ KO mice were purchased from

Jackson laboratory¹¹. *Mme* KO mice were used as negative controls⁵⁷. C57BL/6J and ICR mice were used as zygote donors and foster mothers. C57BL/6J mice were also used for backcrossing with *Ensa* KO mice. App^{NL-F} mice harbor the humanized sequence of A β , and the Swedish (KM670/671NL) and Iberian (I716F) mutations, while App^{NL-G-F} mice harbor the Arctic (E693G) mutation in addition to the humanized sequence of A β , and Swedish (KM670/671NL) and Iberian (I716F) mutations as previously described¹⁵. Male mice were used in all experiments.

Antibodies

Antibodies used in this research are listed in Supplemental Table 7. The specificity of ENSA antibody was confirmed using the *Ensa* KO mouse.

Primary neurons

Neurons from the cerebral cortex, hippocampal and basal ganglia regions of brains from embryonic day (E) 16-18 C57BL/6Ncr mice were isolated and cultured. Briefly, brains were excised and placed in culture plates (FALCON) containing neurobasal medium (Thermo Fisher Scientific). The aforementioned brain regions were excised by scalpel and treated with 5 ml of 0.25% trypsin solution (Nacalai tesque 32777-44) at 37°C for 15 minutes. 250 µl of 1% DNase I was added by pipette and mixed. Subsequently, centrifugation was performed at 1500 rpm for 5

minutes and 5 ml of Hank's buffered salt solution containing 250 µl of 1% DNase I was added to the pellet and incubated in a water bath at 37°C for 5 minutes. An additional 10 ml of Hank's buffered salt solution was added to the mixture and centrifuged at 1500 rpm for a further 5 minutes. The resulting pellet was added to neurobasal medium with B27 Plus Supplement (Thermo Fisher Scientific 17504044) and 25µM glutamine (Thermo Fisher Scientific 05030-149). The cells were filtered using a cell strainer with 100 µm nylon mesh (Falcon 2360), and seeded on 6- or 96-well plates (Falcon 353046 or Corning 356640). Cortical/hippocampal and basal ganglia neurons were mixed in a 9:1 ratio as co-cultured neurons.

Neprilysin activity

NEP activity measurements were performed on primary neurons after 15-28 days of in vitro (DIV15-28) culture as previously described²⁰. Somatostatin (Peptide institute 4023), TT232 (Tocris 3493), recombinant ENSA (abcam ab92932), recombinant NSG-1 (Creative BioMart NSG1-332H), recombinant NUCKS-1 (Creative BioMart NUCKS1-10956M) and diazoxide (Wako 364-98-7) were added as appropriate concentrations, and cells were incubated for a further 24 hours. Neurons were then incubated with substrate mixture (50 µM suc-Ala-Ala-Phe-MCA (Sigma S8758), 10 nM benzyloxycarbonyl Z-Leu-Leu-Leucinal (Peptide institute 3175-V) and

cOmplete EDTA-Free-Protease inhibitor (Roche Diagnostics 4693132) in 0.2 M MES buffer (pH6.5) with or without Thiorphan (Sigma T6031) for 1 hour at 37°C. Following this, 0.1 mM phosphoramidon (Peptide Institute 4082) and 0.1 mg/ml leucine aminopeptidase (Sigma L-5006) were added, and the reaction mixture was incubated at 37°C for a further 30 minutes. 7-Amino-4-methylcoumarin fluorescence was measured at excitation and emission wavelengths of 380 nm and 460 nm, respectively. Centrifugal 10 and 30 kDa filters (Merck UFC503096, 501096) were used to separate conditioned media obtained from cortical/hippocampal neurons.

Preparation of membrane fractions from brain tissue

Brain tissues were homogenized in Tris-buffer (50 mM Tris pH 8, 0.25 M sucrose, EDTA-free cOmplete protease inhibitor cocktail (Roche Diagnostics 05056489001)) and centrifuged at 3600 rpm and 4°C for 30 minutes. Collected supernatants were centrifuged at 70,000 rpm at 4°C for 20 minutes. Resultant pellets were solubilized in Tris-buffer containing 1% Triton X-100 and incubated on ice for 1 hour before centrifugation at 70,000 rpm at 4°C for 20 minutes. Protein concentrations of membrane fractions in collected supernatant samples were measured by BCA protein assay kit (Thermo Fisher Scientific 23225).

LC-MS/MS analysis

50 mM Ammonium bicarbonate, 10% acetonitrile and 20 mM dithiothereitol were added to the conditioned media and incubated for 30 minutes at 56°C. Samples were then treated with 30 mM iodoacetamide and incubated for 30 minutes at 37°C and digested by incubation with 100 ng/µl trypsin overnight at 37°C. Peptide sequences were determined by Q Exactive Orbital Mass Spectrometers (Thermo Fisher Scientific)⁵⁸. We used Proteome Discoverer Software (Thermo Fishier Scientific) for identification of proteins and peptides. Proteins identified in conditioned media are listed in Table 1.

Preparation for Cas9 and sgRNAs

For synthesis of Cas9 mRNA *in vitro*, plasmid vector pCAG-T3-hCAS-pA (Addgene 48625) was linearized by Sph I, then transcribed with T3 RNA polymerase (Promega) in the presence of Ribo m⁷G Cap Analog (promega) as previously described⁵⁹. The MEGAshortscript T7 (Thermo Fisher Scientific AM1354) and MEGAclear (Thermo Fisher Scientific AM1908) kits were used for *in vitro* transcription of sgRNAs, while the CRISPR Design tool was used for creating sgRNAs⁶⁰. All oligonucleotide sequences used for *in vitro* transcription are listed in Supplemental Table 3.

Microinjection of mouse zygotes

The SpCas9 mRNA (60 ng/µl) and sgRNAs (30 ng/µl) were injected into the cytoplasm of

C57BL/6J zygotes. After incubation at 37°C for 24 hours, embryos developed to the 2-cell-stage were transplanted into host ICR mice.

Off-target analysis

Off-target sites that accepted up to three mismatches were determined by COSMID (<u>https://crispr.bme.gatech.edu/</u>)¹⁹. Target sites were amplified from tail genomic DNA by PCR using the Ex Taq Polymerase kit (Takara RR001A) with primers listed in Supplemental Table 2. Target sequencing was performed using a DNA sequencer (ABI 3730xl).

Genotyping

Genomic DNA was extracted from mouse tail using lysis buffer (100 mM Tris pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 20 μ g/ μ l Proteinase K) and PCR performed using the specific primer set listed in Supplemental Table 6. PCR products were analyzed by MultiNa (Shimadzu) to evaluate the efficiency of the CRISPR-mediated deletion of the *Ensa* gene. Sanger sequencing analyses were conducted using a DNA sequencer (ABI 3730xl).

Western blot analysis

Mouse brains were homogenized with lysis buffer (50 mM Tris pH 7.6, 0.15 M NaCl and cOmplete protease inhibitor cocktail (Roche Diagnostics 11697498001)) using a Multi-bead

shocker MB (Yasui-Kikai). Samples were rotated at 4°C for 1 hour and centrifuged at 15000 rpm for 30 minutes. Supernatants were collected as lysates and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF or nitrocellulose membranes. For detection of ENSA and CTF-APP, membranes were boiled in PBS for 5 minutes, treated with ECL prime blocking buffer (GE healthcare RPN418) for 1 hour and incubated with antibody at 4°C. Dilution ratios of antibodies are listed in Supplemental Table 7. Immunoreactive bands were visualized by ECL Select (GE Healthcare RPN2235) and a LAS-3000 Mini Lumino image analyzer (Fujifilm).

Co-incubation of ENSA and NEP

25 ng/µl of ENSA were co-incubated with 2.5 ng/µl NEP, 0-500 nM A β_{42} (Peptide Institute 4420s) and arctic A β_{42} (Peptide Institute AF-721), 0.1 mM thiorphan, 1 mM phosphoramidon, and 10 mM EDTA at 37°C for 24 hours in 0.2 M MES buffer pH 6.5.

Immunohistochemical analysis

After deparaffinization of paraffin-embedded mouse brain sections, antigen retrieval was performed by autoclaving at 121°C for 5 minutes. Sections were then treated with 0.3% H₂O₂ in methanol to inactivate endogenous peroxidases. Next, sections were rinsed several times with

TNT buffer (0.1 M Tris pH 7.5, 0.15 M NaCl, 0.05% Tween20), blocked for 30 min (TSA Biotin System kit), and incubated overnight at 4°C with primary antibody diluted in TNB buffer (0.1 M Tris pH 7.5, 0.15 M NaCl). Sections were rinsed several times and incubated for 1 hour at room temperature with biotinylated secondary antibody (Vector Laboratories). Next, sections were incubated with HRP-conjugated avidin for 30 minutes and tyramide-enhanced FITC or rhodamine for 10 minutes. Finally, sections were treated with DAPI (Cell Signaling Technology 4083S) diluted in TNB buffer before mounting with PermaFluor (Thermo Fisher Scientific TA-030-FM). Sections were scanned on a confocal laser scanning microscope FV-1000 (Olympus) and a NanoZoomer Digital Pathology C9600 (Hamamatsu Photonics) followed by quantification with Metamorph Imaging Software (Molecular Devices) and Definiens Tissue Studio (Definiens).

Enzyme-linked immunosorbent assay

Mouse cortices were homogenized in TBS buffer (50 mM Tris pH 7.6, 150 mM NaCl, protease inhibitor cocktail) by a Multi-bead shocker (YASUI KIKAI), centrifuged at 70000 rpm for 20 minutes, and supernatants collected as Tris-soluble fractions. Pellets were rinsed with TBS buffer following which 6M guanidine-HCl solution was added and mixed with a Pellet Pestle (KIMBLE). The mixture was then incubated for 1 hour at room temperature. Next, samples were centrifuged

at 70000 rpm for 20 minutes and supernatants collected as guanidine-soluble fractions. Trissoluble fractions and guanidine-soluble fractions were applied to 96-well plates. A β_{40} and A β_{42} levels were measured with the aid of an A β -ELISA kit (Wako 294-62501,294-62601).

Determination of amino acid sequence of NEP-cleaved ENSA

After co-incubation of ENSA and NEP with or without thiorphan, MALDI-TOF analysis was performed using Autoflex speed (BRUKER) to detect the specific fragment of ENSA cleaved by NEP. LC-MS/MS analysis was then performed to determine the specific amino acid sequences. Data from LC-MS/MS analyses are listed in Supplemental Table 4.

SFV injection

WT mice (3 months) were used for this experiment. SFV-NEP vectors (active and inactive forms) were developed previously³⁰. Mice were anesthetized with a triple mixed anesthetic (Domitor 0.3 mg/Kg, Dormicum 4 mg/kg, Bettlefar 5 mg/kg), with SFV then injected into the bilateral hippocampus (stereotaxic coordinates: anteroposterior, -2.6 mm; mediolateral, ± 3.1 mm; dorsoventral, -2.4 mm) in a total volume of 1 µl using a Hamilton syringe (Altair Corporation), at a constant flow rate of 0.1 µl/min using a Legato 130 syringe pump (KD Scientific, Hollistoon, MA). After injection, mice were administrated with Antisedan 3 mg/kg and maintained for 48

hours in cages with free access to food and water.

RNA extraction and semi-quantitative RT-PCR

Total RNA was extracted from the cortex and hippocampus of brains using RNAiso Plus (Takara

9109) according to the manufacturer's protocol. Reverse transcription was performed using

ReverTra Ace (TOYOBO FSQ-301). Primer pairs are listed in Supplemental Table 6. Semi-

quantitative RT-PCR was conducted using a QuantStudio system (Thermo Fisher Scientific).

Diazoxide treatment of mice

Diazoxide was diluted in drinking water and administered to WT and App^{NL-F} mice (10 mg/kg/day). For the short-term treatment, diazoxide was administrated to 3-month-old WT mice for 1 month, while in the long-term treatment, diazoxide was administrated to 15-month-old WT and App^{NL-F} mice for 3 months. After diazoxide treatment for 3 months, mice were subjected to behavioral tests followed by brain dissection.

Open field test

WT mice and App^{NL-F} mice were placed individually in a white noise box for at least 1 hour before starting the test. They were then placed in the middle of an open field maze (600x600 mm) and allowed to explore in the area for 10 minutes. The amount of time that mice spent in the central

region was measured as an anxiety parameter.

Contextual fear conditioning test

Before the start of test, the mice were put in the white noise box for at least 1 hour. Subsequently, the mice were placed into a sound-attenuating chamber and allowed to explore the chamber for 5 minutes. The percent freezing time was measured until mice received an electric shock (7.5mA) to the foot after 4 minutes. As a long-term retention test, the same conditioning experiments were repeated daily for 4 days. The training box was cleaned with water and wiped dry with paper toweling before the next mouse was placed in the chamber. Mice were returned to their cages and provided with free access to food and water.

Human tissues

Brain samples were kindly provided by Dr. John Trojanowski (University of Pennsylvania) in compliance with RIKEN ethics committee guidelines (approval number Wako3 30-4(2)). Other human samples were obtained from Bio Chain and Tissue solutions. All samples are listed in Supplemental Table 5.

Statistics

All data are shown as the mean ±SEM. For comparisons between two groups, data were

analyzed by Student's or Welch's *t*-test. For comparisons among more than three groups, we used one-way analysis of variance (ANOVA) followed by Dunnett's post hoc analysis or Tukey's post hoc analysis. In the contextual fear conditioning test, we used two-way ANOVA followed by Tukey's post hoc analysis. All data were analyzed by Prism7 software (San Diego, CA, USA).

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(T.C.S).

Author Contributions

N.W., T.S., T.C.S. designed this study. N.W and T.S planned the experiments. N.W., N.K., M.T

performed the experiments. N.W analyzed the data and prepared the Figures and Tables. N.W.,

S.H., H.S. and T.C.S wrote and edited the manuscript. All authors provided feedback and agreed

on the final manuscript.

Competing Interests

The authors declare no competing interests.

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Figure legends

Fig.1 Identification of ENSA as a regulator of NEP activity in vitro. a-c, NEP activity after

treatment of co-cultured cells with 1 μM somatostatin or TT232 for 24 hours.

Cortical/hippocampal (Ctx&Hip) neurons (n = 12 wells per treatment), co-cultured neurons (n =

10 wells per treatment), and basal ganglia neurons (n = 8 or 9 wells per treatment) were used. d-

f, NEP activity in co-cultured neurons after the replacement of the culture medium with

conditioned media from Ctx&Hip and basal ganglia neurons treated with 1 µM somatostatin

for 0-6 hours. n = 6-10 wells per treatment in co-cultured neurons. **g-i**, NEP activity in cocultured neurons after incubation with ENSA, NSG-1 and NUCKS-1 recombinant proteins for 24 hours. n = 8-10 wells per treatment in co-cultured neurons. **j-l**, Immunoblotting of ENSA in 3-month-old WT, *Sst₁/Sst₄* dKO and *Srif* KO mice (n = 4 for each group). Values indicated in the graph show ENSA band intensities normalized to that of β -actin. **m-p**, Immunostaining of ENSA in the cortices and hippocampal CA1 and CA3 regions from 3-month-old WT, *Sst₁/Sst₄* dKO, and *Srif* KO mice (n = 6 for each group). Data represent the mean ±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 (one-way ANOVA with Dunnett's post-hoc test).

Fig.2 Elevation of NEP activity in *Ensa* **KO mice. a,** Immunostaining of NEP (Red) and VGAT (Green) from hippocampi of 3-month-old WT and *Ensa* KO mice. Scale bar is 100 μ m in low magnification image and 50 μ m in high-magnification image. **b,** Statistical analysis of NEP immunoreactivity (n = 5 for each group). LM: lacunosum-molecular layer, Omo: Outer molecular layer and MMo: middle molecular layer. **c,** Statistical analysis of co-localized NEP and VGAT signals (n = 5 for each group). **d,** NEP activity in membrane fractions from hippocampi of 3-month-old WT and *Ensa* KO mice (WT: n = 7, *Ensa* KO: n = 6). **e,** Aβ₄₀ ELISA of

hippocampi of 3-month-old WT and *Ensa* KO mice (WT: n = 5, *Ensa* KO: n = 6). **f**, A β_{42} ELISA of hippocampi of 3-month-old WT and *Ensa* KO mice (WT: n = 5, *Ensa* KO: n = 6). g, $A\beta_{42}$ ELISA of hippocampi of 3-month-old Mme KO mice and Ensa/Mme dKO (Mme KO: n = 4, *Ensa/Mme* dKO: n = 5). h,i, Immunostaining of A β (Green), NEP (Red) and DAPI (blue) from 18-month-old App^{NL-F} and App^{NL-F}/Ensa KO mice. Statistical analysis of amyloid plaque area in 18-month-old App^{NL-F} and $App^{NL-F}/Ensa$ KO mice (n = 6 for each group). Scale bar is 100 μ m j, AB42 ELISA of Tris-HCl-buffered saline-soluble (Ts) hippocampal fractions from 18-month-old App^{NL-F} and $App^{NL-F}/Ensa$ KO mice $(App^{NL-F}: n = 7, App^{NL-F}/Ensa$ KO: n = 6). k, A β_{42} ELISA of guanidine-HCl-soluble (GuHCl) hippocampal fractions from App^{NL-F} and App^{NL-F}/Ensa KO mice $(App^{NL-F}: n = 7, App^{NL-F}/Ensa$ KO: n = 6). I, Immunostaining of NEP (Red) and VGAT (Green) in hippocampi of 18-month-old App^{NL-F} and App^{NL-F}/Ensa KO mice. Scale bar is 100 µm in lowmagnification image and 50 µm in high-magnification image. m, Statistical analysis of NEP immunoreactivity (n = 4 for each group). **n**, Statistical analysis of co-localized NEP and VGAT signals (n = 4 for each group). Scale bar is 100 μ m in low-magnification image and 50 μ m in high-magnification image. Data represent the mean ±SEM. *P<0.05, **P<0.01, ****P<0.0001 (Student's or Welch's t-test).

Fig.3 Identification of ENSA as a substrate for NEP. a, Immunoblotting of ENSA incubated with or without NEP and mentioned inhibitors for 24 hours at 37°C. Thio: Thiorphan, Phos: Phosphoramidon. b, Specific peak of full-length of ENSA after incubation with or without NEP and thiorphan. c, Specific peak of cleaved ENSA after incubation with or without NEP and thiorphan. d, Sequence of full-length of ENSA. Arrowheads indicate cleavage site by NEP. e,f, Immunoblotting of ENSA from hippocampi of 6-month-old WT and Mme KO mice. Values indicated in the graph show ENSA band intensities normalized to that of β -actin (n = 5 for each group). g-i, Immunoblotting of NEP and ENSA from hippocampi of 3-month-old WT mice after overexpression of active or inactive mutant NEP by SFV gene expression system. Values indicated in the graph show NEP and ENSA band intensities normalized to that of β -actin (n = 4 for each group). j, Immunostaining of ENSA (Green), NEP (Red) and DAPI (Blue) in CA3 from 3-month-old WT, Ensa KO and Mme KO mice. Scale bar is 50 µm in low-magnification image and 10 µm in high-magnification image. White arrows indicate co-localized signals. Data represent the mean ±SEM. **P*<0.05, *****P*<0.0001 (Student's or Welch's *t*-test).

Fig.4 Increased levels of ENSA in AD model mouse and postmortem brain tissue from patients with AD. a-d, Immunoblotting of ENSA in cortices and hippocampi of 24-month-old WT and App^{NL-F} mice. Values indicated in the graph show ENSA band intensities normalized to that of β -actin (n = 4 for each group). e,f, Immunostaining of ENSA (Red) and A β (Green) in cortex, and hippocampal CA1 and CA3 regions of 24-month-old WT and App^{NL-F} mice (n = 4 for each group). Scale bar is 100 µm. g,h, Semi-quantification of ENSA mRNA levels in cortices and hippocampi of 24-month-old WT and App^{NL-F} mice. Values indicated in the graph show ENSA band intensities normalized to that of G3PDH (n = 4 for each group). i,j, Immunoblotting of ENSA in cortices of healthy controls and AD patients. Values indicated in the graph show ENSA band intensities normalized to that of β -actin (healthy controls: n = 3, AD patients: n = 5). k,l, Immunostaining of ENSA in cortices of healthy controls and AD patients (healthy controls: n = 5, AD patients: n = 6). Scale bar is 500 µm in low-magnification image and 100 µm in highmagnification image. m,n, Immunostaining of ENSA incubated with or without NEP, Thiorphan, A β_{42} and Arctic A β_{42} for 24 hours at 37°C (n = 7-8 for each group). o-r, Immunoblotting of ENSA in cortices and hippocampi of 6-month-old WT and App^{NL-G-F} mice. Values indicated in the graph show ENSA band intensities normalized to that of β -actin (n = 4 for each group). In **,b,d,f,j,l** the

data represent the mean \pm SEM. **P*<0.05, ***P*<0.01, (Student's *t*-test). In **n**, the data represent the mean \pm SEM. ***P*<0.01, *****P*<0.0001 (one-way ANOVA with Turkey's multiple comparison test). Information concerning human samples is given in Supplemental Table 5.

Fig.5 Improvement of Aβ pathology and memory function in App^{NL-F} mice via enhancement of NEP activity by Dz treatment. a, NEP activity after treatment of co-cultured neurons for 24 hours with different doses of diazoxide (Dz) (n = 9-10 for each group). b, NEP activity in membrane fractions from anterior cortex (Ctx), posterior Ctx and hippocampus (Hip) of 4-monthold WT mice treated with or without Dz (n = 6 for each group). c,d, Immunoblotting of NEP in anterior Ctx of 4-month-old WT mice treated with or without Dz. Values indicated in the graph Values indicated in the graph show NEP band intensities normalized to that of β -actin (n = 4 for each group). e, $A\beta_{42}$ ELISA of GuHCl fractions from anterior Ctx and Hip of 4-month-old WT mice with or without Dz (Dz (-): n = 6, Dz (+): n = 7). **f**, A β_{42} ELISA of GuHCl fractions from anterior Ctx and Hip of 6-month-old Mme KO mice with or without Dz (n=8 for each group). g, Freezing ratio of 18-month-old WT and App^{NL-F} mice treated with or without Dz (WT Dz (-): n = 12, WT Dz (+): n = 13, App^{NL-F} Dz (-): n = 10, App^{NL-F} Dz (+): n = 11). h,i, Immunostaining of

Aβ (Green) and NEP (Red) in Ctx, Subiculum and Molecular layer from 18-month-old App^{NL-F} with or without Dz (n = 7 for each group). Scale bar in cortical image = 500 µm and hippocampal image = 200 μ m. **j**, A β_{42} ELISA of GuHCl fractions from cortices and hippocampi of 18-month old App^{NL-F} with or without Dz (n = 8 for each group). k, Immunostaining of NEP (Red) and VGAT (Green) in hippocampi from 18-month old App^{NL-F} with or without Dz. Scale bar is 100 μm in low-magnification image and 50 μm in high-magnification image. j, Statistical analysis of immunoreactivity of NEP (n = 5 for each group). LM: lacunosum-molecular layer, Omo: Outer molecular layer and MMo: middle molecular layer. k, Statistical analysis of co-localized signals of NEP and VGAT (n = 5 for each group). In **a**, the data represent the mean \pm SEM. *P<0.05, ***P<0.001 (one-way ANOVA with Dunnett's post-hoc test). In b, d, e, i, j, l, m, the data represent the mean \pm SEM. *P<0.05, **P<0.01, (Student's *t*-test). In **g**, the data represent the mean ±SEM. On day 3, WT Dz (+) vs App^{NL-F} Dz (-) *P<0.05. On day 4, WT Dz (-) vs App^{NL-F} Dz (-) *P<0.05, WT Dz (+) vs App^{NL-F} Dz (-) **P<0.01, App^{NL-F} Dz (-) vs App^{NL-F} Dz (+) *P<0.05

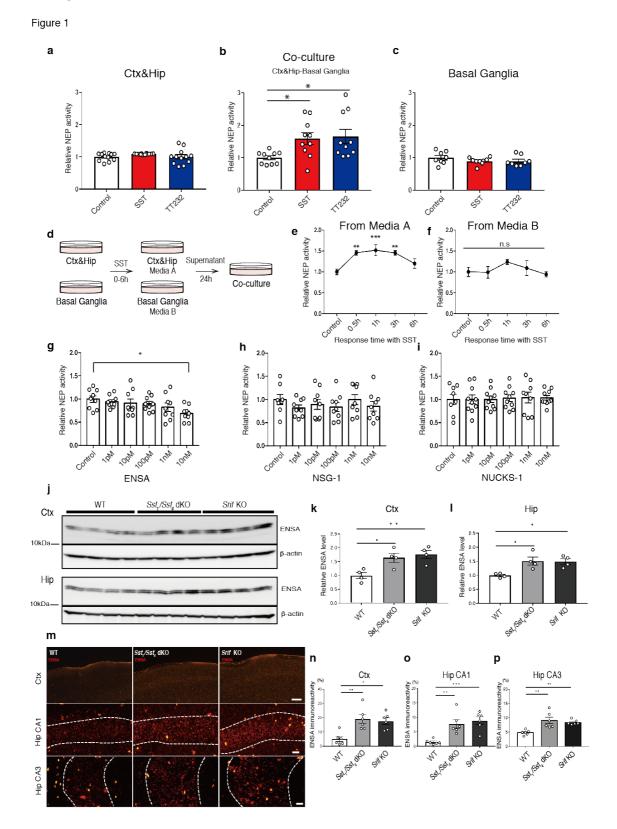
(two-way ANOVA with Turkey's multiple comparison test)

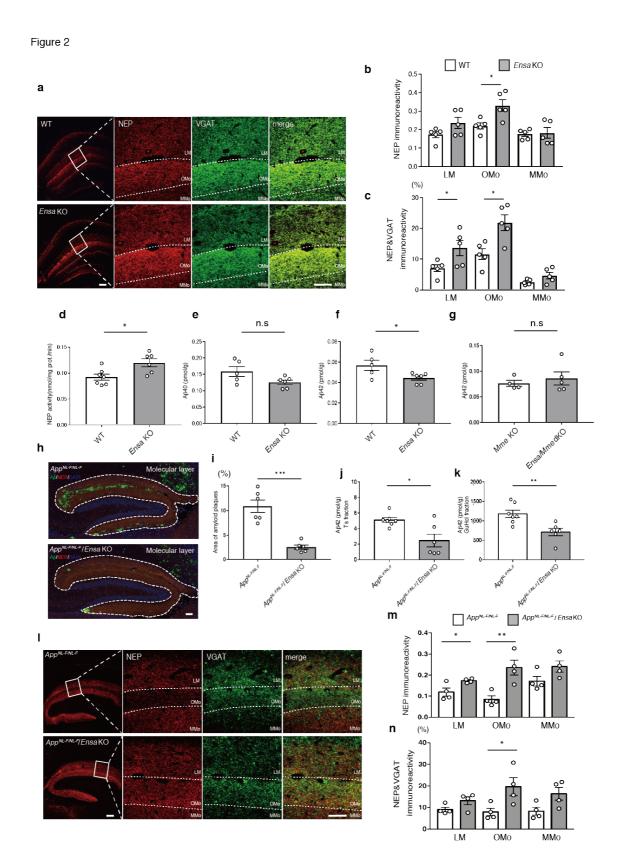
Printery Manual Constant accurate Constraints (PE - 19 V-2 - 1984) 368 9.0 7 9.10 310 1 1 10.122 10.1 Printery Account of Constant accurate Constant Cons	Q913V3 226455 226657 292WL7 29	rain add soluble protein 1 OS=Mus musculus GN=Basp1 PE-1 SV-3 [BASP]. MOUSE] surromodulin OS=Mus musculus GN=GP3 PE-1 SV-1 [NEUM MOUSE] serain, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] erain, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 17 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 15 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] train, type 1 cytosketella 16 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] tealiothinomin 1 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] tealiothinomin 1 OS=Mus musculus GN=KH13 PE-1 SV-1 [NCUSE] tealiothinomin 2 OS=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] teanoriginin fator HTF1 homolog I OS=Mus musculus GN=KH14 PE-1 SV-3 [NCUSE] teanoriginin fator GN=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] teanoriginin fator GN=Mus musculus GN=KH12 PE-1 SV-3 [NCUSE] teanoriginin fator SN=Mus musculus GN=KH12 PE-1 SV-1 [NCUSE] teanoriginin fator SN=Mus	356.46 259.68 447.27 378.20 31.72 537.27 288.16 48.67 496.88 60.08	36.25 33.04 24.02 21.90 24.00 20.17 16.63 19.67	6 8 14 11 1 17 8	16 7 11 18 14 1 25	score 231.84 51.49 37.55 423.10 468.64	5.83 13.66 21.02 21.68	1 2 11 10	8 1 2 15 15	score 382.12 133.72 121.71 234.34	coverage 58.41 11.00 18.06 9.47 15.49	9 2 4 5 7	PSMs 11 3 5 7 7 7	MW(kDa) 22.1 29.6 23.6 48.1 50.1
Prints Matrix bias Control State State Control State	226645 226645 220652 290WL7 290WL7 290WL7 290WL7 290WL7 292WL7	yristorylated alanine-rich C-kinase substrate OS-Mus musculus GN+Marcks PE-1 SV-2- [PA euromodulin OS-Mus musculus GN-Bcapl 3PE 13 Ver 3 - [NEUM MOUSE] eratin, type I cytosketetal 17 OS-Mus musculus GN+Kt12 PE-1 SV=3 - [KLC1, MOUSE] primosin beta-4 OS-Mus musculus GN+Kt12 PE-1 SV=1 - [KLC4, MOUSE] eratin, type I cytosketetal 3 CS-Mus musculus GN+Kt15 PE-1 SV-1 - [KLC5, MOUSE] eratin, type I cytosketetal 5 OS-Mus musculus GN+Kt15 PE-1 SV-1 - [KLC5, MOUSE] eratin, type I cytosketetal 5 OS-Mus musculus GN+Kt16 PE-1 SV=3 - [KLC6, MOUSE] etailohtionein-1 OS-Mus musculus GN+Kt16 PE-1 SV=3 - [KLC6, MOUSE] etailohtionein-1 OS-Mus musculus GN+Kt16 PE-1 SV=3 - [KLC6, MOUSE] etailohtionein-3 OS-Mus musculus GN+Kt62 PE-1 SV=3 - [KLC6, MOUSE] etailohtionein-3 OS-Mus musculus GN+Kt62 PE-1 SV=3 - [XLC6, MOUSE] etailohtionein-3 OS-Mus musculus GN+Kt62 PE-1 SV=3 - [XLC6, MOUSE] etailohtionein-3 OS-Mus musculus GN+Bt7E-1 SV=1 - [MT], MOUSE]	356.46 259.68 447.27 378.20 31.72 537.27 288.16 48.67 496.88 60.08	36.25 33.04 24.02 21.90 24.00 20.17 16.63 19.67	6 8 14 11 1 17 8	7 11 18 14 1 25	51.49 37.55 423.10 468.64	5.83 13.66 21.02 21.68	1 2 11 10	1 2 15 15	133.72 121.71 234.34	11.00 18.06 9.47	2	3 5 7	29.6 23.6 48.1
PHOP: Neuroscienti 0 5-the manualis 01-the ph Pel SV-1 [PER MODE] 298.44 314 8 11 97.83 336 2 2 313.41 00000 Menti, Nic L 4 Contract, 01-the manualis 01-the Pel SV-1 [PER MODE] 317.2 340.0 1<	P06837 P20065 P20065 P20065 P20105	euromodulin OS-Mus musculus GN-Gap43 PE-1 SV=1 - (NEUM_MOUSE) errain, type I cytosketel 17 CS-Mus musculus GN-Htt1 PE-1 SV=3 - [KL17, MOUSE] errain, type I cytosketel 37 CS-Mus musculus GN-Htt1 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN-Htt1 PE-1 SV=1 - [TVH MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] errain, type I cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] erain, type II cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] eraint, type II cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] eraint, type II cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [KL12, MOUSE] eraint type II cytosketel 3 CS-Mus musculus GN+Ht12 PE-1 SV=3 - [ST14, MOUSE] eraint type II cytosketel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [ST12, MOUSE] eraint type II cytosketel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [SUCC, MOUSE] eraint type II Cytoshetel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [SUCC, MOUSE] eraint type II Cytoshetel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [SUCC, MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [SUCC, MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SodI PE-1 SV=3 - [SV1-2 - [SUCC, MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SodI PE-1 SV-3 - [SUCC MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SOdI PE-1 SV=3 - [SUCC MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SOdI PE-1 SV-3 - [SUCC MOUSE] Eraint type II Cytoshetel 3 CS-Mus musculus GN+SOdI PE-1 SV-3 - [SUCC MOUSE] Eraint type II Cytoshetel 3 CS-Musculus type II CYtoshetel SV=3 - [SUCC MOUSE] Eraint type II Cytoshetel 3 CS-Mus	259.68 447.27 378.20 31.72 537.27 288.16 48.67 496.88 60.08	33.04 24.02 21.90 24.00 20.17 16.63 19.67	8 14 11 1 17 8	18 14 1 25	37.55 423.10 468.64	13.66 21.02 21.68	2 11 10	2 15 15	121.71 234.34	18.06 9.47	4	5	23.6 48.1
Operative Name Section Section 2012 (Section 2014) Section 2014	920/U/2 QGIPX2 920065 922022 9222X1 922X2 922X1 922X2 922X1 920282 920292 92029 9	eratin, type I cytoskeletal 17 OS=Mus musculus GN=Kt12 /PE-1 SV=3 - [KL17, MOUSE] tymosin beta-4 OS=Mus musculus GN=Kt12 /PE-1 SV=1 - [KL25, MOUSE] tymosin beta-4 OS=Mus musculus GN=Kt15 /PE-1 SV=1 - [KL25, MOUSE] teratin, type I cytoskeletal 5 OS=Mus musculus GN=Kt15 /PE-1 SV=3 - [KL16, MOUSE] tetaliothionein-1 OS=Mus musculus GN=Kt16 /PE-1 SV=3 - [KL16, MOUSE] tetaliothionein-1 OS=Mus musculus GN=Kt16 /PE-1 SV=3 - [KL16, MOUSE] tetaliothionein-1 OS=Mus musculus GN=Kt16 /PE-1 SV=3 - [KL17, MOUSE] tetaliothionein-3 OS=Mus musculus GN=Kt16 /PE-1 SV=3 - [KL26A, MOUSE] tetaliothionein-3 OS=Mus musculus GN=Kt16 /PE-1 SV=3 - [KL26A, MOUSE] tetaliothionein-3 OS=Mus musculus GN=Kt6a /PE-1 SV=3 - [SOCA, MOUSE] tetaliothionein-3 OS=Mus musculus GN=Kt6a /PE-1 SV=3 - [S	447.27 378.20 31.72 537.27 288.16 48.67 496.88 60.08	24.02 21.90 24.00 20.17 16.63 19.67	14 11 1 17 8	18 14 1 25	423.10 468.64	21.02 21.68	11 10	15 15	234.34	9.47		7	48.1
Color Control, Spin L globaled 42 (2) E-Man mutual gli-incit 2 PE-13 (VI-1) (VI-2), POSE 1 2020 Throme II and the H-3 C-Man mutual gli-incit 2 PE-11 (VI-1), POSE 1 2020 Throme II and the H-3 C-Man mutual gli-incit 2 PE-11 (VI-1), POSE 1 2020 Throme II and the H-3 C-Man mutual gli-incit 2 PE-11 (VI-1), POSE 1 2020 Throme II and the H-3 C-Man mutual gli-incit 2 PE-11 (VI-1), POSE 1 2020 Throme II and the H-3 C-Man mutual gli-incit 2 PE-12 (VI-1), POSE 1 2020 POSE 1 <	QcIFX2 Q20065 Q922U2 Q922U2 Q922V1 P02798 P02802 P28184 Q9CQH7 P70633 Q9C036 Q64387 P07309 P26350 P07309 P26350 Q61762 Q8006439 P70663 Q9D036 Q9D036 Q9D045 Q60764 Q80765 Q60764 Q80765 Q607644 Q60764 Q60764 Q60764 Q60764 Q60764 Q60764 Q60764 Q60764 Q607764 Q60764 Q6077764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q607764 Q6077764 Q607764 Q607764 Q607764 Q607764 Q607764 Q6077764 Q6077764 Q60777777777777777777777777777777777777	ratin, type I ortosketedi 42 OS=Mus musculus GN=Krt42 PE-1 SV=1 - (KLC42, MOUSE) privosin beta-40 S=Mus musculus GN=Mist Met SI SV=1 - (TM4 MOUSE) ratin, type I ortosketedi 14 OS=Mus musculus GN=Krt5 PE-1 SV=1 - [IAC5 MOUSE] etailothionein-1 OS=Mus musculus GN=Krt5 PE=1 SV=2 - [MT2, MOUSE] etailothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [MT2, MOUSE] etailothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [MT3, MOUSE] etailothionein-1 OS=Mus musculus GN=Krt68 PE=1 SV=3 - [IAC36, MOUSE] etailothionein-3 OS=Mus musculus GN=Krt68 PE=1 SV=3 - [IAT3, MOUSE] etailothionein-3 GS=Mus musculus GN=Krt68 PE=1 SV=1 - [IMT3, MOUSE] etailothionein-3 GS=Mus musculus GN=Krt68 PE=1 SV=2 - [SOE0, MOUSE] etailothionein-3 GS=Mus musculus GN=Krt68 PE=1 SV=3 - [SOE0, MOUSE] Etailothionein-3 GS=Mus muscul	378.20 31.72 537.27 288.16 48.67 496.88 60.08	21.90 24.00 20.17 16.63 19.67	11 1 17 8	14 1 25	468.64	21.68	10	15			7	,	
179000 Thymoin beh-i G-Ma macula GN-TMBA PE-1 SV-1. (TPN MODE] 312 140 1 </td <td>P20065 Q922U2 Q92ZX1 P02798 P02802 P50446 P28184 Q9CQH7 P08228 Q64387 P07309 P26350 P02535 P06710 P70663 Q9D038 P0C649 P0C649 P0C649 P0C649 P0C649 P0C649 P60840 P26339 Q6IF26 P60941 Q8VED5 Q6N814 Q6P814</td> <td>hymosin beta-1 OS=Mus musculus GN=Tmsb4x PE=1 SV=1 - [TYB4_MOUSE] eratin, type I (rotosketela 15 OS=Mus musculus GN=Kt15 PE=1 SV=1 - [I2CS_MOUSE] eratin, type I (rotosketela 16 OS=Mus musculus GN=Kt16 PE=1 SV=3 - [KIC16_MOUSE] etallothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [MT1_MOUSE] etallothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT1_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT3_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT3_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 OS=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 GN=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 GN=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE]</td> <td>31.72 537.27 288.16 48.67 496.88 60.08</td> <td>24.00 20.17 16.63 19.67</td> <td>1 17 8</td> <td>1 25</td> <td></td> <td></td> <td></td> <td></td> <td>100.95</td> <td>15.45</td> <td>/</td> <td>+ '</td> <td></td>	P20065 Q922U2 Q92ZX1 P02798 P02802 P50446 P28184 Q9CQH7 P08228 Q64387 P07309 P26350 P02535 P06710 P70663 Q9D038 P0C649 P0C649 P0C649 P0C649 P0C649 P0C649 P60840 P26339 Q6IF26 P60941 Q8VED5 Q6N814 Q6P814	hymosin beta-1 OS=Mus musculus GN=Tmsb4x PE=1 SV=1 - [TYB4_MOUSE] eratin, type I (rotosketela 15 OS=Mus musculus GN=Kt15 PE=1 SV=1 - [I2CS_MOUSE] eratin, type I (rotosketela 16 OS=Mus musculus GN=Kt16 PE=1 SV=3 - [KIC16_MOUSE] etallothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [MT1_MOUSE] etallothionein-1 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT1_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT3_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=1 - [MT3_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 OS=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 GN=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE] etallothionein-3 GN=Mus musculus GN=Sod1 PE=1 SV=2 - [SOC0_MOUSE]	31.72 537.27 288.16 48.67 496.88 60.08	24.00 20.17 16.63 19.67	1 17 8	1 25					100.95	15.45	/	+ '	
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PRADE Metalliphonenin LG-Mus macular, GM-VHI FPE 1 VI - 1 (MT 1400, FE) Perator State State<	P02802 P50446 P28184 Q9CQH7 P08228 Q64387 P07309 P26350 P02535 P60710 P70663 Q9D0J8 P0C649 P60840 P26339 Q6IF26 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	etallothionein-1 OS-Mus musculus GN=MtJ PE=1 SV=1 - [MT1_MOUSE] etallothionein-3 OS-Mus musculus GN=Mt3 PE=1 SV=1 - [MT3_MOUSE] etallothionein-3 OS-Mus musculus GN=Mt3 PE=1 SV=1 - [MT3_MOUSE] mascription factor BTF3 homolog of GS-Mus musculus GN=Br3H PE=2 SV=1 - [BT3L4_MOU upperoxide dismutase [Cu-2n] OS=Mus musculus GN=Br3H PE=2 SV=1 - [SOC_MOUSE] erponoclogetin OS-Mus musculus GN=PG0 PE=2 SV=1 - [SOC_MOUSE]	496.88 60.08					19.67		1	******	0.00	,		6.1
Piester Exercitin, Specif Constrained Coll + 2014 (1994) Piester	P50446 P28184 Q9CQH7 P08228 Q64387 P07309 P26350 P02535 P60710 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P70663 Q9D038 P06740 P10663 Q9D038 P06740 P10663 Q9D038 P06740 P10663 Q9D038 Q60741 Q80824 Q698814 Q02257	eratin, type II cytosketetal 6A 05=Mus musculus GN+Krt6a PE-1 SV=3 - [K2GA_MOUSE] etailothionein-3 O5=Mus musculus GN+M2 PE = 1 SV=1 - [MT3_MOUSE] arascription factor BTF3 homolog 4 O5=Mus musculus GN=Btf3I4 PE=2 SV=1 - [BT3L4_MOU uperoxide dismutase [Cu-2n] O5=Mus musculus GN=SodLF1 SV=2 - [SODC_MOUSE] erponociceptin O5=Mus musculus GN=Picot PE=2 SV=1 - [PNCC_MOUSE]	60.08	10.17		-				1	62.32	19.67	1	1	6.0
Pathel Metalethonen-B - Go-Has muzuka GN-HB2 Pie-1 SV-1 - (PTI A) MOLES 0.60 1.25 1 1 0.00 9933 127 Vieldon Transmittion factor PID Hower (SA Pitta Filler A) VI-1 (PTI A) MOLES 1.31 1.43 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.44 1.43 1.43 1.44 1.43 1.44 1.43 1.43 1.44 1.43 1.44 1.43 1.43 1.44 1.43 1.43 1.44 1.43 1.43 1.44 1.43 1.43 1.44 1.43 1.43 1.44 1.43 1.43 1.43 1.43 1.44 1.43	P28184 Q9CQH7 P08228 Q64387 P07309 P26350 P02535 P60710 P70663 Q9D038 P00C649 P00C649 P00C649 P00C649 P00C49 P0	etailothionein-3 OS=Mus musculus GN=M3 PE=1 SV=1 - [MT3_MOUSE] ranscription factor BTF3 homolog 4 OS=Mus musculus GN=Btf3l4 PE=2 SV=1 - [BT3L4_MOU uperoxide dismutase [Cu-2n] OS=Mus musculus GN=Sot1 PE=1 SV=2 - [SODC_MOUSE] repronocideptin OS=Mus musculus GN=Pnoc PE=2 SV=1 - [PNOC_MOUSE]			15	20						10.13	8	8	59.3
Open Deck Transcription factor BT3 homolog 4 GS-Max muscular GN-BPI FF = 2 SV = 1 (BT4, MQZ Number Supervised and	P08228 Q64387 P07309 P26350 P02535 P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q61FZ6 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	ranscription factor BTF3 homolog 4 OS=Mus musculus GN=Btf3l PE=2 ŚV=1 - [BT314, MOU uperoxide dismutase [Cu-Zn] OS=Mus musculus GN=Sod1 PE=1 SV=2 - [SODC_MOUSE] repronociceptin OS=Mus musculus GN=Pnoc PE=2 SV=1 - [PNOC_MOUSE]	24.10	17.65	1	1		0.00			59.93	17.65	1	1	7.0
TPR2E Supervised domatase (Lo Zn) GS=Max maculas GN=Solf Fer 1 SV-2 (EOC PAUSE) 77.10 15.8 2 2 40.8 8.4 1 1 89.77 84.4 04307 Preprioration GS=Max maculas GN=Thy TE P1 SV-1 (TIME VAUSE) 33.65 14.44 14.4 10.54 12.1 77.10 11.8 77.10 12.8 77.10 11.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 77.10 12.8 1 1 77.10 12.8 1 1 12.8 1 1 18.1 1 1 18.1 1 12.8 1 1 13.8 1 1 18.1 1 1 18.3 1 1 18.3 1 1 18.3 1 1 18.3 1 1 18.3 1 1 18.3 1	Q64387 P07309 P26350 P02535 P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	uperoxide dismutase [Cu-Zn] OS=Mus musculus GN=Sod1 PE=1 SV=2 - [SODC_MOUSE] repronociceptin OS=Mus musculus GN=Pnoc PE=2 SV=1 - [PNOC_MOUSE]		16.46	1	1									17.3
Ophits Preproduceding On-Plus maculas GN-Purp E 19:V -1 (TPM C, MOLSE) 33.65 14.44 1 I Image: Control of Control of Control of Control of Control On C	P07309 P26350 P02535 P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IF26 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	repronociceptin OS=Mus musculus GN=Pnoc PE=2 SV=1 - [PNOC_MOUSE]		15.58	2	2	40.26	8.44	1	1	59.57	8.44	1	1	15.9
P0729 Transflurento GS-Max muculus GN-TD FE-1 SV-1 (TTM, MOUSE) 2004 10.20 1 0.00	P26350 P02535 P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	rangthurstin OS-Mus musculus CN-Th RE-1 SV-1 FTTHY, MOUSEI	33.65	14.44	1	1					24.44	14.44	1	1	20.9
P2035 Prothymosin alpha GS=Mas muzquiss GH=/bm #F=1 SY=2 (PTM, MQUSE] 2000 1.5.1 3 4 175.41 12.61 2 2 775.51 VEXIS Mortal DS=Mas musculus GH=/bth FE=1 SY=1 (ACTE MODEE] 135.80 7 11 32.80 0.1 1 127.06 11.3 VEXIS Mortal DS=Mas musculus GH=/bth FE=1 SY=1 (ACTE MODEE] 159.80 2.18 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.88 1 1 22.66 11.85 1 1 15.84 1 1 15.84 1 1 15.84 1 1 15.84 1 1 15.84 1 1 1 15.84 1 1 1 15.84 1 1 1 1 1 1 1 1	P02535 P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257		20.04	10.20	1	1		0.00				4.08	1	1	15.8
month abs constraint abs constraint abs constraint abs constraint abs constraint abs abs constraint abs abs </td <td>P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257</td> <td>rothymosin alpha OS=Mus musculus GN=Ptma PE=1 SV=2 - [PTMA_MOUSE]</td> <td></td> <td></td> <td>3</td> <td></td> <td></td> <td></td> <td>2</td> <td></td> <td></td> <td>12.61</td> <td>2</td> <td>2</td> <td>12.2</td>	P60710 P70663 Q9D038 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	rothymosin alpha OS=Mus musculus GN=Ptma PE=1 SV=2 - [PTMA_MOUSE]			3				2			12.61	2	2	12.2
Physical Actin, Cytoplasmic 1, Clo-Max musculus, GN-Path (Per, 1, Vi-1, (ACT, MUOSE) 39:5 2.67 1 1 2.28 8.00 1 1 34.11 14 Profile SPARL, September 1, Sema Musculus, GN-Path (Per, 2, Vi-1, 12) 6.6 6. 11.8 1 2 2.06 11.8 1 2.06 11.8 1 2.06 11.8 1 1 2.06 11.8 1 1 2.06 11.8 1 1 2.06 11.8 1 1 2.06 11.8 1 1 1.0 0.00 1 1 1.0 0.00 1 1 1.0 0.00 1 1 1.0 0.00 1 1 1.0 0.00 1 1.0 0.00 1 0.00 1 0.00 0.0	P70663 Q9D0J8 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	eratin, type I cytoskeletal 10 OS=Mus musculus GN=Krt10 PE=1 SV=3 - [K1C10_MOUSE]			7	11			7	10		12.11	8	10	57.7
Corport Farsthymodin Co-Mus musculus GN-Murp Pers Per J SV-1 - [PMS, MUSE] 66.86 1.88 1 1 2.2.97 1.8.8 1 1 2.2.97 00000 Polyabilith Co-Mus musculus GN-Murp Per J SV-1 - [RDS, MUSE] 19.98 1.5.7 5 6 3.0.0 1 1 1.1.80 1 1 1.1.80 1 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1 1.1.80 1.1.80 1 1.1.80 1.1.80 1 1.1.80 1.1.80 1.1.80 1 1.1.80 1.1.80 1 1.1.80	Q9D0.38 P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P814 Q02257	ctin, cytoplasmic 1 OS=Mus musculus GN=Actb PE=1 SV=1 - [ACTB_MOUSE]					32.89		1	1		1.60	1	1	41.7
Provide Digital - Biologia - Maxim secular, GH-Lab PE-2, SV-1: [VBL, MOUSE] Image: Provide Digital - Comparison of Comparison - Compar	P0CG49 P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	PARC-like protein 1 OS=Mus musculus GN=Sparcl1 PE=2 SV=3 - [SPRL1_MOUSE]										3.23	1	1	72.2
Oppose Oppose<	P60840 P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q6P8I4 Q02257	arathymosin OS=Mus musculus GN=Ptms PE=1 SV=3 - [PTMS_MOUSE]	66.86	11.88	1	2				1	32.69	11.88	1	1	11.4
Tep2339 Chromogranin A GS-Mus musculus GN-Erbg PF=1 SV=1 - [CICLB MOUSE] 192.67 97.2 5 6 6.609 2.33 1 1 11.544 5.41 Grinz Meratin, Dpe II ortisolettal ID GS-Mus musculus GN-Erbg PE = 1 SV=1 - [CICLB MOUSE] ID ID 76.21 ID 76.23 71 ID 66.70 90.99 72.33 ID 76.23 76.23 76.23 72.33 ID 76.23	P26339 Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	olyubiquitin-B OS=Mus musculus GN=Ubb PE=2 SV=1 - [UBB_MOUSE]					0.00	11.80	1	1					34.3
Control Karadin, Spe II. Considered II. ID GS-MBM. musculas GN-MCV7 PE-1 SV-1- [FQC1E_MOLSE] 219.79 524 6 100 359.42 9.82 7 111 106.97 5.75 GWHON Construction GS-MMS musculas GN-MCV79 PE-2 SV-2- [CC79_MOLSE] 175.08 9.4 100 776.51 8.10 6 7 120.99 7.73 9.2 GWHON Keratin, Spe II. Constellart TO GS-MMS musculas GN-MCV79 PE-2 SV-2- [CC79_MOLSE] 17.00 9.42 9 100 776.51 8.10 6 7 120.99 7.73 9.2 GWHON Keratin, Spe II. Constellart TO GS-MMS musculus GN-MCVD PED PE1 SV-1- [FQL7] 28.33 6.99 1 1 6 7 1.0 7.0 9.2 7.0 1.0 7.0 9.2 7.0 1.0 7.00 9.00 1 1 1 1.0 7.00 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	Q6IFZ6 P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	<pre>ipha-endosulfine OS=Mus musculus GN=Ensa PE=1 SV=1 - [ENSA_MOUSE]</pre>													13.3
Open Display Constructions Constructions <thconstructions< th=""> Constructi</thconstructions<>	P60041 Q8VED5 Q6NXH9 Q6P8I4 Q02257	hromogranin-A OS=Mus musculus GN=Chga PE=1 SV=1 - [CMGA_MOUSE]							1			5.83	3	3	51.8
Operation Serietin, hype II optoskeled 79 OS-Mus musculus GN-Kr/27 PE-1 SV-1 (CT 20, MOUSE) 17.68 9.42 9 10 17.531 8.10 6 7 120.99 7.1 060MM Kerstin, hype II optoskeled 73 OS-Mus musculus GN-Kr/27 PE-1 SV-1 (F 825.3 9.83 1 1	Q8VED5 Q6NXH9 Q6P8I4 Q02257		219.79	5.42	6	10	350.42	9.62	7	11		5.42	5	5	61.3
Generatin, Spe II. Crobasched in 73 GS-Mix musculus GN-May Results. GN-May Res 1 Syst 1, 122, 29, 28 6 10 298.41 5.94 3 8 177.53 9.2 GWBH PETS proteinty is signed-analysis. GN-May Results. GN-May Res 1 Syst 1, 121, 28, 31 8.9 1 <	Q6NXH9 Q6P8I4 Q02257												1	1	12.7
Operator PEST professive signal-containing nuclear protein GS-Mis musculus SN+Prop PE=1 Sy+1. [C 28:53 899 1 1	Q6P8I4 Q02257	eratin, type II cytoskeletal 79 OS=Mus musculus GN=Krt79 PE=2 SV=2 - [K2C79_MOUSE]								7		7.16	6	6	57.5
-02227 Junction plakagolden 0.5 = Mus musculus GN=Jup Fe-1 SV=3 - [PLK, MOLES] 73.7 4 4 65.39 6.57 1 1 P10957 Timotion 0.5 = Mus musculus GN=Mus PL = 1 SV=3 - [TLK], MOLSE] 2 2.2 2.39 2.2 P12979 Lycopme C-1 0.5 = Mus musculus GN=Mus PL = 1 SV=3 - [TLK], MOLSE] 2.400 8.05 1 </td <td>Q02257</td> <td>eratin, type II cytoskeletal 73 OS=Mus musculus GN=Krt73 PE=1 SV=1 - [K2C73_MOUSE]</td> <td></td> <td></td> <td></td> <td></td> <td>298.41</td> <td>5.94</td> <td>3</td> <td>8</td> <td>177.53</td> <td>9.28</td> <td>6</td> <td>7</td> <td>58.9</td>	Q02257	eratin, type II cytoskeletal 73 OS=Mus musculus GN=Krt73 PE=1 SV=1 - [K2C73_MOUSE]					298.41	5.94	3	8	177.53	9.28	6	7	58.9
P1090 Thioredoan 0.5=Mus musculus GN=Tor, PE=1 SV=3 - [THI0, MOJSE] Cols 65.29 6.57 1 1 P1290 Lyoover, C1 0.5=Mus musculus GN=TyPE=1 SV=1. [VC1, MOJSE] 24.90 8.05 1 1		EST proteorytic signal-containing nuclear protein OS=Mus musculus GN=Pcnp PE=1 SV=1 - [28.53				05.50	4.02	2	2	27.20	2.42		1	19.0 81.7
P1287 Lysozyme C-1 GS-Mis musculus GN-Huryl PE-I SV-1 [VLZ], MOUSE] Col 73.44 8.11 1 1 67122 Statim GS-Mis musculus GN-Huryl PE I SV-2 : TMNI MOUSE] 2.400 8.05 1 1 0 <td>L 1002A</td> <td>ancuon piakogiobin OS=Mus musculus GN=Jup PE=1 SV=3 - [PLAK_MOUSE]</td> <td>/3.1/</td> <td>0.1/</td> <td>4</td> <td>4</td> <td></td> <td></td> <td></td> <td></td> <td>27.39</td> <td>2.92</td> <td>1</td> <td>1</td> <td>81.7</td>	L 1002A	ancuon piakogiobin OS=Mus musculus GN=Jup PE=1 SV=3 - [PLAK_MOUSE]	/3.1/	0.1/	4	4					27.39	2.92	1	1	81.7
1 1	D17807					-									11.7
201732 Pared immunopolabilin-like type 2 receiptor beta OS-Mus musculus GN-PHID PE-1 SV-1- [PIL88 MOUSE] - - 1333 7.59 1 1 - - 1333 7.59 1 1 - - 1333 7.59 1 1 - - 1333 7.59 1 1 - - 4 49.57 7.7 200717 Transcription elongation factor: A protein-like 5 GS-Mus musculus GN+Fr2 PE-1 SV-1-1 [F022 MOUSE] 18.34 6.67 4 4 - 59.25 33.3 201715 Secretor Secretor Secretor Secretor PE-1 SV-1-1 [F022 MOUSE] 18.69 6.53 1 1 0.00 - 10.0 10.1 10.00 10.0 10.00 <		tathmin OS=Mus musculus GN=Stm1 PE=1 SV=1 - [LYZ1_MOUSE]	24 90	8.05	1	1	73.04	0.11	1	1		0.00			16.8
COUNT: Keratin, type II. droxsteletal 2 and CG=Hux musculus GN=KY72 PE=2 Sys1-1; CI22D, MOUSE] 146.24 741 6 6 79.34 3.03 2 4 95.76 7.7 C007CT Transpitol endopation fact A protein-like 5 GS=Mus musculus GN=Tosito FE=2 Sv1-1; CI22D, MOUSE] 183.4 6.67 4 4 0 95.25 3.3 C00317 Seratin Musculus GN=Sign Seration		aired immunoglobulin-like type 2 recentor beta OC-Mue musculus CN-Dilyb DC-1 CV-1 [D]			1	1	13.93	7.59	1	1		0.00			25.2
CQCCT Transcription elongation factor A protein-like 5 OS-Mus musculus GN+Fr2aB /FE-2 SVI-1. [TC 2123 7.00 1 1 0 9572 CQDSTJ Secret musculus GN-Secret SQC PE-1 SVI-1. [SCG2 MOUSE] 88-34 6.67 4 4 0 9572 CQDSTJ Secret SQC PE-1 SVI-1. [SCG2 MOUSE] 18-69 6.55 9 10 164.08 5.37 6 8 13.174 6.00 0 0.00 0 10.00 0.00 0 10.00 0.00		eratio, type II ovtoskeletal 2 oral OS=Mus musculus GN=Krt76 PF=2 SV-1 - TK220 MOLICET	146.24	7.41	6	6					95.76	7.58	7	7	62.8
Construction Secretorigramin - 2GS=Mus musculus GN=Seq2 PE-1 SV=1 - [SCG_MOUSE] 88:34 6.97 4 4 6 6 9 10 104:08 5.37 6 8 13.77 6 6 10 104:08 5.37 6 8 13.77 6 6 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 10 0.00 10 <th< td=""><td></td><td>ranscription elongation factor A protein-like 5 OS=Mus musculus GN=Treal5 PE=2 SV=1 - FT</td><td>21.23</td><td></td><td>1</td><td>1</td><td>10.01</td><td>5105</td><td></td><td></td><td>3500</td><td>7100</td><td>-</td><td></td><td>22.0</td></th<>		ranscription elongation factor A protein-like 5 OS=Mus musculus GN=Treal5 PE=2 SV=1 - FT	21.23		1	1	10.01	5105			3500	7100	-		22.0
Q3175 Keratin, hype II optaskeletal 2 spidermal CS=Mus musculus GN=Kr2 PE=1 SV=1- [F02E MOUSE] 665 9 10 16408 5.37 6 8 131.74 6.00 0 00 P2826 MRACKS*related protein GS=Mus musculus GN=Inst PE=1 SV=1- [INS]. MOUSE] 16.69 6.50 1 1 0.00 0 197.6 6.44 7 16 25.44 6.44 8 15 56.11 6.44 7 16 25.44 6.44 8 15 56.11 6.44 7 16 25.44 6.44 8 15 56.11 6.44 7 16 25.44 6.44 8 15 56.11 6.44 7 16 25.44 6.44 1 1 2 73.38 4.4 P10746 Reptdity-froxtyl do-trans isoonerase A OS=Mus musculus GN=Mappa PE=1 SV=2 - [FKBP2, IQUOSE] 123.45 4.44 2 0.00 2 77.38 5.3 Q60400 Postod SD=Shvs musculus GN=Mappa PE=1 SV=2 - [FKBP2, IQUOSE] 152.54 4.44 2 10 1		ecretographin-2 OS=Mus musculus GN=Scn2 PE=1 SV=1 - [SCG2_MOUSE]	88.34		4	4					59.25	3.40	2	2	70.6
P2865 MARCS-related protein OS=Mus musculus GN=Marck11 PE-1 SV=2 (MRP. MOUSE] 18.69 6.00 1 1 0.00 1972 1000 P0132 Insulus GN=Inst ISV=1 (MS) Solution SI 6.04 8 15 56.11 6.44 7 16 22.74 6.6 P0130 Kerathy, type II cytoskeletal 1CS-Mus musculus GN=Hot PE=1 SV=1 (KC72, MOUSE] 102.92 5.54 5.55 1 1 1 2.86 5.54 5.54 5.54 5.54 5.54 5.54 5.54 <td></td> <td>eratin, type II cytoskeletal 2 epidermal OS=Mus musculus GN=Krt2 PE=1 SV=1 - [K22E_MOU</td> <td></td> <td></td> <td></td> <td></td> <td>164.08</td> <td>5.37</td> <td>6</td> <td>8</td> <td></td> <td>6.65</td> <td>8</td> <td>9</td> <td>70.9</td>		eratin, type II cytoskeletal 2 epidermal OS=Mus musculus GN=Krt2 PE=1 SV=1 - [K22E_MOU					164.08	5.37	6	8		6.65	8	9	70.9
OPU132 Insulin-1 OS=Mus musculus GN=Inst PE 1 SV=1. [INS1_MOLES] 000	P28667	ARCKS-related protein OS=Mus musculus GN=Marcksl1 PE=1 SV=2 - [MRP_MOUSE]	18.69	6.50	1	1		0.00				0.00			20.2
PM140 Reration, type II. protoskeletal 1.05 - Mus musculus GN=krt1 PE=1 SV=1 (EGC1, MOUSE] 409.24 6.44 8 15 56.17 6.44 7 16 22.74 6.64 060929 Metanization on moter 1.05 - Mus musculus GN=Mus (PE=1 SV=1 (EGC1, MOUSE) 103.02 55.54 5.55 5.57 4 6 53.09 1.73 1 2 73.88 4.0 06/P174 Peptidy-(prol) di-strans isomerase A CS=Phals musculus GN=Phyle P18 F=1 SV=2 - (PEN, MOUSE) 131.79 52.57 2 2 0.00 C 77.58 5.5 06/000 Multime expression factor 2 OS=Mus musculus GN=Phyle P18 F=1 SV=2 - (PEN, MOUSE) 131.79 52.57 2 2 0.00 C 77.58 5.1 06/000 Phyle diversion factor 2 OS=Mus musculus GN=Pkilts P198 F=1 SV=2 - (PEN, POUSE) 2.366 4.40 4.50 1 1 C C 78.53 4.44 2 2.00 344.2 1 18.12 4.44 07074 Secondogranin .05 S=Mus musculus GN=CALP PE=1 SV=2 - (SCG1, MOUSE] 12.54 4.43 2 2	P01325	sulin-1 OS=Mus musculus GN=Ins1 PE=1 SV=1 - [INS1 MOUSE]		0.00				0.00			19.76	6.48	1	1	12.2
Operator Number of the second se	P04104		409.24	6.44	8	15	561.17	6.44	7	16	252.49	6.44	7	9	65.6
17:274 Peptidy-provide starts isomerase AOS = Mus musculus GN = Pop A FE 1 SV = 2 (PPLA MOUSE) 23.03 5.49 1 1 C 28.07 5.57 Q00254 Myeline personin factor QS = Mus musculus GN = Pop A FE 1 SV = 1 (PPLA MOUSE) 21.03 5.25 2 2 0.00 77.55 5.25 Q00254 Myeline personin factor QS = Mus musculus GN = Pop A FE 1 SV = 1 (PPLA MOUSE) 0.60 5.13 1 1 0.00 77.55 5.25 Q00244 Peptidy-provid vist mission constrait 1 OF Mus macrait CPN entropic Mouse) 0.60 5.13 1 1 0.00 77.55 5.25 Q00200 ProSAAS OS = Mus musculus GN = Pop Lin S = 1 SV = 1 (PPLA MOUSE) 75.53 4.44 2 0.00 77.55 4.47 4.47 4.467 4.482 4.03 2 2 0.00 77.55 4.44 2 1 81.12 4.44 P1014 Secretogranin - 3.05 -Mus musculus GN = Pop Lin S = 1 SV = 1 (FURA) MOUSE 12.54 4.43 2 2 0.00 77.55 4.41 1 0.00 3.02 2		euron-specific protein family member 1 OS=Mus musculus GN=Nsg1 PE=2 SV=3 - [NSG1_M	55.54		1	1									20.9
P1724 Peptidy-provid vis-trans isomerase A 05=Hus musculus GN+Ppia PE-1 SV-2 (PPIA, MOLEE) 23.63 5.49 1 1	Q6IME9	eratin, type II cytoskeletal 72 OS=Mus musculus GN=Krt72 PE=3 SV=1 - [K2C72_MOUSE]	102.92	5.77	4	6	53.09	1.73	1	2	73.38	4.04	3	3	56.7
Operator	P17742	eptidyl-prolyl cis-trans isomerase A OS=Mus musculus GN=Ppia PE=1 SV=2 - [PPIA_MOUSE]	23.63	5.49	1	1					28.97	5.49	1	1	18.0
Opcimie Peptidy-provid seturals isomerase R4873 OS=Mus musculus GN=Rba3 PE=1 SV=2-[FRUP3, 2 246 941 1 1 0 4412 (0702W) POSAMS OS=Mus musculus GN=Abs, PE=1 SV=2-[FSUR3, MOUSE] 73553 4447 4457 4457 4467 4457 1 1 0.00 431.2 444 (0702W) POSAMS OS=Mus musculus GN=Abs, PE=1 SV=2-[FSG3, MOUSE] 125.24 4.44 2 20 344.25 4.44 2 10 813.12 44. P1041 Secretogrannin -1 OS=Mus musculus GN=Chep PE=1 SV=2-[FSG3, MOUSE] 125.24 4.43 2 2 0.00 77.83 44 P1041 Secretogrannin -1 OS=Mus musculus GN=Chep PE=1 SV=1-1 [FSG3, MOUSE] 10 0.00 3.92 2 0.00 2.2 0.00 1 1 0.00 1 1 1.0 0.00 2.2 0.00 2.2 0.00 2.0 0.00 2.0 0.00 2.0 0.00 2.0 0.00 2.0 0.00 2.0 0.00 2.0 0.00 2.0 0.00		yelin expression factor 2 OS=Mus musculus GN=Myef2 PE=1 SV=1 - [MYEF2_MOUSE]						0.00			77.58	5.25	2	2	63.3
(PQ0W) ProSAAS OS=hus musculus GN=PeckIn PE=1 SV=3-1[RAB_MOUSE] 44.67 1 1 0.00 81.2 44.47 PV0724 Secure Journal Common Communication Secure Journal Common C		uclear ubiquitous casein and cyclin-dependent kinase substrate 1 OS=Mus musculus GN=Nucl			1	1									26.3
IP0724 Serum albumin 0S=Mus musculus GN=Alb PE=1 Sy=3- [ALBU_MOLSE] 735.53 4.44 2 20 344.25 4.44 2 10 819.12 4.44 P10414 Secretogranin-1 CS=Mus musculus GN=Chep PE=1 Sy=2- [SGG]_MOLSE] 125.24 4.43 2 2 0.00 72.58 4.44 P10415 Secretogranin-1 CS=Mus musculus GN=Chep PE=1 Sy=2- [SGG]_MOLSE] 125.24 4.43 2 2 0.00 72.58 4.44 P1780 Secretogranin-1 CS=Mus musculus GN=Perd PE=1 Sy=1- [SGG]_MOLSE] 1.0 0.00 3.52 2.2 1.0 1 1.000 1.0 1.0 0.00 3.52 2.2 1.0 1.0 1.0 0.00 3.52 2.2 1.0 1.0 0.00 3.2 2.2 2.0 1.0 1.0 0.00 3.0 1.0 3.0 1.0 3.0 1.0 3.0 1.0 3.0 1.0 1.0 0.00 2.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0		eptidyl-prolyl cis-trans isomerase FKBP3 OS=Mus musculus GN=Fkbp3 PE=1 SV=2 - [FKBP3_			1	1									25.1
P1014 Secretogranm 1 CS =Mus musculus CN = Chip PE = 1 Sv = 2 (SCG, MOXE) 125 At A 4.43 2 2 0.00 P278 8 4.43 2 2 0.00 P278 8 4.43 2 2 0.00 P278 8 4.43 2 2 P <		roSAAS OS=Mus musculus GN=Pcsk1n PE=1 SV=2 - [PCSK1_MOUSE]		4.65	1	1						4.65	1	1	27.3
PP3780 Secretogranin-3 CS=Mus musculus CN=Scq3 PE-1 SV=1 - [\$CG3 MOUSE] 23.88 4.03 2 2 0 0 P17870 Percendonin 1 CS=Mus musculus CN=Cq3 PE-1 SV=1 - [\$FCG3 MOUSE] 1 0.00 3.92 2 2 1 1 1 0.00 3.92 2 2 1 1 1 0.00 3.92 2 2 1 1 1 0.00 3 <td< td=""><td></td><td></td><td></td><td>4.44</td><td>2</td><td></td><td>344.25</td><td></td><td>2</td><td>10</td><td></td><td>4.44</td><td>2</td><td>24</td><td>68.6</td></td<>				4.44	2		344.25		2	10		4.44	2	24	68.6
P27200 Percentedoant-1 GS=Mus musculus GN=Pridu PE=1 Sy-1-[PROX, MOUSE] P <t< td=""><td></td><td></td><td></td><td></td><td></td><td>2</td><td></td><td>0.00</td><td></td><td></td><td>72.58</td><td>4.43</td><td>2</td><td>2</td><td>77.9</td></t<>						2		0.00			72.58	4.43	2	2	77.9
P12182 Alpha endlase OS=Mus musculis GN=End IP=1 [V+3] : EINOA_MOUSE] 0 0.00 3.92 2 2 PP2731 Ver5-Specific debulghinges BRC3G OS=Mus musculis GN=End IP=2 [V+3] : EINOA_MOUSE] 3.60 1 1 0.00 3.92 2 2 PP2731 Ver5-Specific debulghinges BRC3G OS=Mus musculis GN=End PE-2 [V+1] : EINOA_MOUSE] 3.60 1 1 3.160 3.61 1 2.855 3.64 1 1 0.00 2.2 0.00 P20205 Precemplain OS=Mus musculis GN=Perink PE-1 SV+1 - [DESP, MOUSE] 3.60 3 3 3.31 3.37 7.7 7 0.00 C68US Limbic system-associated membrane protein OS=Mus musculis GN=VerDPM OUSE] 3.77 3.22 2 0.00 24.28 1.1 P07355 Annexin ZS-Mus musculis GN=VerDPA = 2 - [ANXA2_MOUSE] 3.77 3.22 2 0.00 24.28 1.4 P07355 Annexin ZS-Mus musculis GN=VerDPA = 2 - [ANXA2_MOUSE] 3.77 3.22 2.6 0.00 24.28 1.4 P07354 Annexin ZS-Mus musculis GN=VerDPA = 2			23.88	4.03	2	2	20.52	4.00							53.3 22.2
PM272 Lys-63-specific deubiquitase BRC236 OS=Mus musculus GN=Brc3 PF=2 SV=1 - [BRC3 MC 2485 3.44 1 1 0.00 P20205 Ponetyphilm OS=Mus musculus GN=Brc3 PF=2 SV=1 - [BRC MOUSE] 3.602 3.64 1 1 1.60 3.66 1 1 2.65 3.35 EV0205 Description Comparison Semantic SV 3.62 3.66 1 1 3.66 1 1 2.65 3.35 EV0505 Description SV Semantic SV 3.664 3.23 1 1 2.65 3.26 1 1 3.66 1 1 2.65 0.00 2.42 0.00 2.42 0.00 2.42 0.00 2.42 1 1 1 1 1 2.65 1 1 1 1 1 1 1.63 2.65 1 1 1 1 1 1 1 1 1 1.63 2.62 1 1 1 1 1 1 1 1<															47.1
Process Process Safe 1 1 31.60 3.6 1 1 28.55 33.55 SPG57D Demoksphalin CS=Mus musculus GN=Perit SV=1 (PEN, MOUSE) 100.83 0.94 3 3 18.377 2.71 7 7 0.00 C8057D Demoksin CS=Mus musculus GN=PET SV=1 (PEN, MOUSE) 100.83 0.94 3 3 18.377 2.71 7 7 0.00 C80102 Limbic system-associated membrane protein CS=Mus musculus GN=Lamp PE-1 SV=1 - (LNA 36.44 3.23 1 1 0.00 24.29 1.1 P07305 Annekin A2 CS=Mus musculus GN=Vm EQD EP1 SV=3 - IMKGB2, MOUSE] 0.54 2.66 1 1 0.00 24.62 1.1 0.00 24.62 1.1 0.00 1.0 0.01 1.0 0.01 2.65 1.1 0.02 2.65 1.1 0.02 2.66 1.0 1.0 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 </td <td></td> <td></td> <td>24.05</td> <td>2.44</td> <td></td> <td></td> <td>0.00</td> <td></td> <td>2</td> <td>2</td> <td></td> <td></td> <td></td> <td></td> <td>33.3</td>			24.05	2.44			0.00		2	2					33.3
EP0557 Desmoplakin GS-Mis musculus GNI-Dap PE-1 SV-1-1; (DESP MUSE] 100.83 0.94 3 3 183.32 2.71 7 <td></td> <td></td> <td></td> <td></td> <td>-</td> <td>1</td> <td>21.60</td> <td></td> <td>1</td> <td>1</td> <td>26.22</td> <td>3.36</td> <td>1</td> <td>1</td> <td>31.0</td>					-	1	21.60		1	1	26.22	3.36	1	1	31.0
C98U0 Limbic system-associated membrane protein OS=Mus musculus GN+Lisamp PE-1 SV=1 - [LSAN 36.84 323 1 1 0 24.29 1.1 P20125 Vinemin GS=Nus musculus GN+VinepE SV=3 - [LYGAN MOUSE] 77.66 3.22 2 0.00 24.29 1.1 P20125 Vinemin A2 OS=Mus musculus GN+VinepE 2F=1 SV=3 - [LYGAN MOUSE] 77.66 3.22 2 0.00 24.29 1.1 P20125 Vinemin A2 OS=Mus musculus GN+VinepE 2F=1 SV=3 - [LYGRE MOUSE] 0.54 2.86 1 1 1.1 <td< td=""><td></td><td>eemonlakin OS-Mus musculus GN-Den DE-1 SV-1 - [DESD_MOLISE]</td><td></td><td></td><td></td><td>3</td><td></td><td></td><td></td><td></td><td>20.55</td><td>0.00</td><td>1</td><td>-</td><td>332.7</td></td<>		eemonlakin OS-Mus musculus GN-Den DE-1 SV-1 - [DESD_MOLISE]				3					20.55	0.00	1	-	332.7
272013 Winnethin OS=Musi musculus GN+Um PE=1 SV=3 - (VIME_ MOUSE] 3776 322 2 2 0.00 24.29 1 79735 Anneu AD OS=Musi musculus GN+Emp ZE=1 SV=2 - (ANAQ2 MOUSE] 3776 322 2 2 0.00 24.29 1 79735 Anneu AD OS=Musi musculus GN+Emp ZE=1 SV=2 - (ANAQ2 MOUSE] 26.69 2.955 1 1 79936 Anneu AD OS=Musi musculus GN+Emp ZE=1 SV=2 - (ANAQ2 MOUSE] 2.669 1 1 44.62 22. 09443 Phosphadter optidy/inpriadrese to 25-Musi musculus GN+Emp ZE=1 SV=2 - (EQ ZE) 4.66 2.469 1 1 44.62 22. 09443 Phosphadter optidy/inprist GN=Mus musculus GN+Emp ZE=1 SV=2 - (EQ ZE) 2.69 1 1 44.62 22. 2.46 1 1 0.00 2.47 1.00 0.01 2.45 2.40 1 0.00 2.45 2.40 1 1 0.00 2.01 0.00 2.01 1 1 0.00 2.02 1 1 0.00 2.02 1 1		mbic system-associated membrane protain OS-Mus musculus GN-Learn DE-1 SV-1 - [LSA					105/57	2.0 2	,	,		0.00		-	38.1
Protosis Anmesin A2 OS=Mus musculus GN=4ma2 PE=1 SV=2 - [AX0A2_MOUSE] 0 2660 2.95 1 1 19961 High mobility group profile B2 OS=Mus musculus GN=Hmp2 PE=1 SV=3 - [HX6B2_MOUSE] 0.954 2.86 1 1 13.62 2.2 09809 Libiquifin carboxyl-terminal hydrolase isozyme L1 OS=Mus musculus GN=Hmp2 PE=1 SV=3 - [CD3_MOUSE] 0.954 2.86 1 1 41.62 2.2 09809 Libiquifin carboxyl-terminal hydrolase isozyme L1 OS=Mus musculus GN=Hoth 2N=2 - [GD3_MOUSE] 0.00 23.45 2.40 1 1 0.0 19858 Giverabre delyhdrog-anbexed SS-Mus musculus GN=Hompk PE=1 SV=1 - [IVA 2.88 1 1 0.0 0.0 1.0 0.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.0 1.0 0.		imentin OS=Mus musculus GN=Vim PE=1 SV=3 - [VIME_MOLISE]	37.76					0.00			24.29	1.50	1	1	53.7
Pioses High mobility group protein B2 OS=Mus musculus GN=HmpD2 PE=1 SV=3 - [HMGB2, MOUSE] 30.54 2.68 1 1 41 41.52 22.2 Q9404 Phosphatidate qridyHupmafreaze 2.0S=Mus musculus GN=GN=LDE ISV=3 - [FL 28.8] 2.69 1 1 41.62 2.2 41.62 2.2 0.05 2.345 2.40 1 41.62 2.2 0.05 0.05 1.0 41.62 2.2 0.05 0.05 1.0 0.0 0.05 0.05 0.05 1.0 0.0 </td <td>P07356</td> <td>nnevin A2 OS=Mus musculus GN=Anxa2 PE=1 SV=2 - [ANXA2_MOUSE]</td> <td></td> <td></td> <td></td> <td></td> <td>26.60</td> <td>2.95</td> <td>1</td> <td>1</td> <td></td> <td></td> <td>-</td> <td></td> <td>38.7</td>	P07356	nnevin A2 OS=Mus musculus GN=Anxa2 PE=1 SV=2 - [ANXA2_MOUSE]					26.60	2.95	1	1			-		38.7
Comparing Comparing <thcomparing< th=""> <thcomparing< th=""> <thc< td=""><td>P30681</td><td></td><td>30.54</td><td>2.86</td><td>1</td><td>1</td><td></td><td></td><td></td><td></td><td>13.62</td><td>2.86</td><td>1</td><td>1</td><td>24.1</td></thc<></thcomparing<></thcomparing<>	P30681		30.54	2.86	1	1					13.62	2.86	1	1	24.1
Openergy Ubiquitin carboxy-terminal hydrolase isozyme L1 OS=Mus musculus GN=dph IP E1 SV= 1 [0 28.81 2.69 1 1 0 <td>Q99L43</td> <td>hosphatidate cytidylyltransferase 2 OS=Mus musculus GN=Cds2 PE=1 SV=1 - [CDS2 MOUSE</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>2.70</td> <td>1</td> <td>1</td> <td>51.3</td>	Q99L43	hosphatidate cytidylyltransferase 2 OS=Mus musculus GN=Cds2 PE=1 SV=1 - [CDS2 MOUSE										2.70	1	1	51.3
P1658s Glyceraddehyde-sphosphate dehydrogenase OS=Mus musculus GNI=Gagdh PE-1 SV=2 - (G2P MOLSE) 0.00 23.45 2.40 1 1 0.01 P16597 Heleropenous nuclear information (So=Mus musculus GNI=Gagdh PE-1 SV=2 - (G2P MOLSE) 0.00 23.45 2.40 1 1 0.01 P15979 Heleropenous nuclear information (So=Mus musculus GNI=HarpApe Mol SS) 8.12 2.38 1 1 7 1.1 7		biguitin carboxyl-terminal hydrolase isozyme L1 OS=Mus musculus GN=Uchl1 PE=1 SV=1 - [28.81	2.69	1	1						0.00			24.8
Phi179 Heterogeneous nuclear ribonucteoprotein K OS=Mus musculus GN=Hmmpk PE-1 SV=1. (HNR) 38.12 2.38 1 1 <		lyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=Gapdh PE=1 SV=2 - [G3F	_MOUSE]	0.00			23.45	2.40	1	1		0.00			35.8
P1149 Heat shock protein HSP 90-bed 05-Mix musculus GN=Hsp0ab1 PE-1 SV=3 - [HS90 MOUL 52.00 22.1 1 1 207077 Amphiphysin OS-Mix musculus GN=Mp90ab1 PE-1 SV=3 - [HS90 MOUL 3.149 2.19 1 1 207077 Amphiphysin OS-Mix musculus GN=AmphiPE-1 SV=1 - [HMPH MOUSE] 3.149 2.19 1 1 207077 Amphiphysin OS-Mix musculus GN=Amph PE-1 SV=1 - [HMP MOUSE] 15.57 2.20 1 3 0.00 2.42 209079 Hetoprenous nuclear monotomologonoron linke protein 2.05 - Mix musculus GN=Amphi PE-1 SV=1 - [HZ14 1 0.00 2.42 Q09079 Hetoprenous nuclear monotomic Nuclearontom linke protein 2.05 - Mix musculus GN=Hermpu2 PE + SI2.44 1.88 1 1 0.00 2.42 Q09079 Hetoprotein 14 OS-Mix musculus GN=Hermpu2 PE + SI2.44 MOUSE] 2.58 1.80 1 1 0.00 2.759 1.49 2.59 1.20 2.579 1.20 2.579 1.20 2.579 1.30 2.579 1.30 2.579 1.30 2.579 1.30 2.579 1.30 2.579 1.30		eterogeneous nuclear ribonucleoprotein K OS=Mus musculus GN=Hnrnpk PE=1 SV=1 - [HNR	38.12		1	1									50.9
QPTOP2 Amphiphysin OS=Mus musculus GNI=Amph PE=1 SV=1 - [AMPH MOUSE] 31.49 2.19 1 1 QPDS18 Protein FAM227B OS=Mus musculus GNI=Amph PE=1 SV=1 - [ZPE MOUSE] 16.57 2.07 1 3 0.00 2.4 QPDF18 Protein FAM227B OS=Mus musculus GNI=Fam227b PE=2 SV=3 - [F227B_MOUSE] 16.57 2.07 1 3 0.00 2.4 QPDF19 Hetergeneous nuclear ribonucleoproten Unike protein 2 OS=Mus musculus GNI=Hrmpul2 PE = SV=3 - [F227M_IMOUSE] 1 1 0.00 2.4 QPOPT0 Hetergeneous nuclear ribonucleoproten Unike protein 2 OS=Mus musculus GNI=Hrmpul2 PE = SV=3 - [F214_IMOUSE] 5.24 1.88 1 1 0.00 2.4 QPOPT0 Hetergeneous nuclear ribonucleoproten Unike protein 2 OS=Mus musculus GNI=Hrmpul2 PE = SV=3 - [F214_IMOUSE] 5.24 1.88 1 1 0.00 2.4 QPOPT0 Houtine bet=5 Abin OS=Mus musculus GNI=Hrub5 PE = 1 YUE = JTEBE MOUSE] 2.55 1.80 1 1 2.759 1.4 QPOPUA Tublim bet=5 Abin OS=Mus musculus GNI=Hrub5 PE = 1 YUE = JTEBE MOUSE] 2.558 1.80 1 1 1 1 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>43.85</td> <td>1.26</td> <td>1</td> <td>1</td> <td>42.97</td> <td>1.26</td> <td>1</td> <td>1</td> <td>119.4</td>							43.85	1.26	1	1	42.97	1.26	1	1	119.4
090518 Protein FAM22278 OS=Mus musculus GN=Fam2270 PE=2 \$V=3 - [F2278_MOLSE] 15.57 2.47 1 3 0.00 2.42 0000PID Hetergeneous nuclear mitority influe protein 2 CS=Mus musculus GN=Hermpil/2 PE 5.24 1.88 1 1 0.00 2.42 000PID Hetergeneous nuclear mitority influe protein 2 CS=Mus musculus GN=Hermpil/2 PE 5.24 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 0.00 2.42 1.88 1 1 1 0.00 2.42 1.88 1 1 1 1 1 1 1 1 1 1<						1									83.2
Q00PB Heterogeneous nuclear ribonucleoprotein L-like protein 2 OS=Mus musculus GN=Hnmpul2 PE 52.24 1.88 1 1 0.4 Q90701 Apoptosis facilitator Bd-2-like protein 14 OS=Mus musculus GN=Bd1214 PE-1 SV=1. FB2.14 MOUSE] 27.59 1.4 P99024 Tubulh beta-5 chain OS-Mus musculus GN=Bd1214 PE-1 SV=1. FB2.14 MOUSE] 27.59 1.4						1									75.0
OPOTO Apoptosis facilitator Bd-2-like protein 14 OS=Mus musculus GN=Bd2114 PE=1 SV=1 - [B2114 MOUSE] P99024 Tubulin beta-5 chain OS=Mus musculus GN=Tubb5 PE=1 SV=1 - [TB85 MOUSE] 26.58 1.80 1 1		rotein FAM227B OS=Mus musculus GN=Fam227b PE=2 SV=3 - [F227B_MOUSE]									0.00	2.07	1	1	62.7
Popole Tubuin beta-5 chain OS=Mus musculus GN=TubbS PE=1 SV=1 - TERES_MOUSE] 265.8 1.80 1 1		eterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Mus musculus GN=Hnrnpul2 PE	52.24	1.88	1	1		_		_	27.55	0.00			84.9
		poptosis facilitator Bci-2-like protein 14 OS=Mus musculus GN=Bcl2l14 PE=1 SV=1 - [B2L14]	MOUSE]	1.00			-	_			27.59	1.83	1	1	37.0
		ubulin beta-5 chain US=Mus musculus GN=Tubb5 PE=1 SV=1 - [TBB5_MOUSE]					10.01	1.70		,	26.07	1.79			49.6
Prosso 100ulin alpha-4A Cani OS=Mus musculus GN=10aP4 PE=1 SV=1 1184A_MOUSE] 30.*** 1.79 1 2 10.91 1.79 1 1 20.87 1		ubulin alpha-4A chain OS=Mus musculus GN=Tuba4a PE=1 SV=1 - [TBA4A_MOUSE]				2	10.91	1.79	1	1	20.87	1.79	1	2	49.9 94.1
		eat snock / u kua protein 4 US=Mus musculus GN=Hspa4 PE=1 SV=1 - [HSP74_MOUSE]				1 7	26.12	1.64	,	,	296.16	1.64	-	10	94.1
		H3 domain-containing protein 21 US=Mus musculus GN=Sh3d21 PE=2 SV=1 - [SH321_MOU	2 85.21				26.13		1	1	286.16	0.00	1	18	60.3 54.3
Q7TPCI Conreodesmosin OS=Mus musculus GN=Cdsn PE=2 SV=2 - [CDSN_MOUSE] 28.45 1.60 1 1 0.00 0.01 P35350 Perovisioner proliferation-activated receptor delta OS=Mus musculus GN=Paperd PE=2 SV=1 - [PPARD_MOU 0.00 21.71 1.5		omeouesmosm OS=MUS MUSCUIUS GN=LOSN PE=2 SV=2 - [CDSN_MOUSE]			1	1					21.71	0.00	1	1	54.3 49.7
		eroxisome promerator-activated receptor detta OS=Plus musculus GN=Ppard PE=2 SV=1 - [P ollad-coll domain-containing protein 93 OS=Mire musculus CN=Code02 DE=2 CV=1 - [CCD02	MOLICE?	0.00		-	-	0.00		_		1.59	1	1	49.7
(2/108) Colled-coil domain-containing protein 93 OS=Mus musculus GM=CatCet39 PE=2 SV=1 = 1 (CD39) GMOSE 1000ESE 10.50 1 1 38.70 1 1 1 1 64.68 1.56 1 1 38.70 1 1 38.70 1 1 38.70 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			OUSE	1 56	1	1	64.69	1 56	1	,		1.59	1	1	86.7
		myolu bela A4 protein OS=MUS MUSCUlus GN=App PE=1 SV=3 - [A4_MOUSE] ecentor-tune turosine-protein phosphatase zeta OS=Mus musculus CN=Dtoral DS=1 CV=1 - 5			2	2			1	1		0.52	1	1	254.2
		eceptor-type tyrosme-protein prosphatase zeta OS=Mus musculus GN=PtpTz1 PE=1 SV=1 - [alevatanin_1 OS=Mus musculus GN=Cletn1 DE=1 SV=1 - [CCTN1_MOUSE3		1.34	-	-	33.02	0.52	1	1		1.33	1	1	254.2
						-					23.73	1.55	1	1	108.8
Q61147 Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2 (CERU, MOUSE) 1.22 1.23 2 2 4 Q61495 Desmojeln-1-alpha GS=Mus musculus GN=Dsga PE=2 SV=2 (DSG1A, MOUSE) 25.57 1.23 1 1 0.00		emonlein_1_alpha_OS=Mus_musculus_GN=Den1a_PE=2_SV=2[UEKU_MOUSE]						0.00							121.1
												0.00			72.4
		ndonlasmin OS=Mus musculus GN=Hsn90h1 PE=1 SV=2 - [ENPL_MOUSE1	42.74								18.31	1.00	1	1	92.4
Protein psic		eticulon-4 OS=Mus musculus GN=Rtn4 PE=1 SV=2 - [RTN4_MOUSE]				-		0.00			10.51	1.00	-	-	126.5
Comparing Redscruder Octoberging 22471 0.00 1 1 P20357 Microtubule-associated protein 2 05=Mix musculus GN=Map2 PEri 15V=2 - [MTAP2_MOUE5] 1913 0.66 1 1		icrotubule-associated protein 2 OS=Mus musculus GN=Man2 PF=1 SV=2 - [MTAD2_MOLICE]													120.5
Procouncie-association protein 2 US=Prius musculus GN=Prapiz Pt=1 SP=2 - [INTIPZ_PMODE] 39-13 0-00 1 1 QRGGR3 Long protease homolog, mitochondrial OS=Nus musculus GN=Prapiz Pt=2 - [LONM_MOL 0-00 1 1 1															199.0
Qeouss Lon protease nomolog, micocininal us=mus musculus GN=Cong_Pice1 sV=2 · [LOWP_mic_] 000 003 1 1 2 24.27 0.0				0.60		1					24.27	0.60	1	1	105.8
POUCY Complement C3 OS=Plus musculus GN=C3 Pt=1 Sy=3 - [LO3_PMOUSE] 21.48 0.60 1 1 2 24.27 0.00 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		omprement CS OS-MUS MUSCUlus GN-CS FE-1 SV=3 - [CO3_MOUSE]		0.54	1	1					27.21	0.00	1	1	186.4
		aural call adhacion molecula L1 OS=Mus musculus GN=L1cam DE=1 SV=2 - [INSKK_MOUSE]			1	1				_	30.68	0.48	1	1	144.8
		inc finger protein castor homolog 1 OS=Mus musculus GN=C1call FE=1 SV=1 - [LICAll_MOUSE	SE1	0.00								0.40	1	1	191.1
246-0112 Zink Imper protein castor nomolog 1 OSE-mus musculus (N=24.25 × 3 × 3 ± 1 CA32.2 + MOUSE) 0000 000 000 000 000 000 000 000 000	D811D2	nkvrin repeat domain-containing protein 26 OS=Mus musculus GN=Apkrd26 PE-1 SV-2 - [A	27.70	0.38	1	1		0.00			0.00	0.00	-	· ·	191.1
Qentuz Anitymin repeat domain-containing protein as OS=mus modulus on=anit/dzo PE=1 SV=2 - [Al 27/0 0.36 1 0.00 0 O60811 Reelin OS=Mus modulus SN=anit/dzo PE=1 SV=2 - [Al 27/0 0.3847 0.29 1 1 0.00 0	060841	eelin OS=Mus musculus GN=Reln PE=1 SV=3 - [RELN_MOUSE]	33.47		1	1		0.00				0.00			387.2
		rotein piccolo OS=Mus musculus GN=Pclo PE=1 SV=4 - [PCL0_MOUSE]		0.00				0.00			19.47	0.18	1	1	550.5

Table1. Proteins identified in LC-MS/MS analysis of conditioned media

ist;/Sat, dKO	Control				+SST				+TT232				
Q91XV3 Brain acid soluble protein 1 OS=Mus musculus GN=Basp1 PE=1 SV=3 - [BASP1_MOUSE]	score 216.17	coverage 46.02	peptide 5	PSMs 6	score 253.04	coverage 51.77	peptide 6	PSMs 8	score 237.87	coverage 46.02	peptide 5	PSMs 6	MW(kDa) 22.1
P08228 Superoxide dismutase [Cu-Zn] OS=Mus musculus GN=Sod1 PE=1 SV=2 - [SODC_MOUSE]	122.39	23.38	3	3	105.38	24.68	3	4	148.60	24.68	3	5	15.9
P06837 Neuromodulin OS=Mus musculus GN=Gap43 PE=1 SV=1 - [NEUM_MOUSE]	130.16	19.82	3	4	150.53	19.82	3	5	158.96	24.67	4	5	23.6
P02798 Metallothionein-2 OS=Mus musculus GN=Mt2 PE=1 SV=2 - [MT2_MOUSE]	24.08	19.67	1	1	48.88	19.67	1	1					6.1
P02802 Metallothionein-1 OS=Mus musculus GN=Mt1 PE=1 SV=1 - [MT1_MOUSE]	55.98	19.67	1	1	63.77	19.67	1	1	42.90	19.67	1	1	6.0
Q9QXV0 ProSAAS OS=Mus musculus GN=Pcsk1n PE=1 SV=2 - [PCSK1_MOUSE]	96.81	18.60	3	3	64.01	10.47	2	2	17.35	18.60	3	3	27.3
P28184 Metallothionein-3 OS=Mus musculus GN=Mt3 PE=1 SV=1 - [MT3_MOUSE]					48.20	17.65	1	1 6	59.43	17.65	1	1	7.0
Q9QWL7 Keratin, type I cytoskeletal 17 OS=Mus musculus GN=Krt17 PE=1 SV=3 - [K1C17_MOUSE] Q9Z2K1 Keratin, type I cytoskeletal 16 OS=Mus musculus GN=Krt16 PE=1 SV=3 - [K1C16 MOUSE]	175.37 123.37	8.78	5	6	189.10 189.63	13.63	6	6	231.72 306.07	15.01	/	8	48.1 51.6
Q6IFX2 Keratin, type I cytoskeletal 10 OS=Mus musculus GN=Krt42 PE=1 SV=3 - [K1C42_MOUSE]	123.37	13.50	6	7	148.55	9.07	5	5	128.48	15.55	7	7	50.1
Q922U2 Keratin, type II cytoskeletal 42 OS=Mus musculus GN=Krt5 PE=1 SV=1 - [K2C5_MOUSE]	274.04	13.10	10	13	238.84	11.72	8	9	206.09	10.34	7	10	61.7
P70663 SPARC-like protein 1 OS=Mus musculus GN=Spard1 PE=2 SV=3 - [SPRL1_MOUSE]	111.28	10.00	4	4	116.05	10.15	4	4	165.05	15.38	7	8	72.2
P50446 Keratin, type II cytoskeletal 6A OS=Mus musculus GN=Krt6a PE=1 SV=3 - [K2C6A_MOUSE]	266.68	10.13	8	11	229.41	10.67	7	8	212.89	11.39	7	10	59.3
Q64387 Prepronociceptin OS=Mus musculus GN=Pnoc PE=2 SV=1 - [PNOC_MOUSE]	27.74	14.44	1	1	33.87	14.44	1	1	63.70	14.44	1	1	20.9
P26350 Prothymosin alpha OS=Mus musculus GN=Ptma PE=1 SV=2 - [PTMA_MOUSE]	182.72	13.51	2	4	257.78	12.61	2	3	285.13	13.51	3	6	12.2
P26339 Chromogranin-A OS=Mus musculus GN=Chga PE=1 SV=1 - [CMGA_MOUSE]	294.99 34.78	10.58	7	8	231.32 75.91	8.42 11.88	5	6	222.38 23.95	12.96 11.88	7	8	51.8 11.4
Q9D038 Parathymosin OS=Mus musculus GN=Ptms PE=1 SV=3 - [PTMS_MOUSE] P02535 Keratin, type I cytoskeletal 10 OS=Mus musculus GN=Krt10 PE=1 SV=3 - [K1C10_MOUSE]	283.46	11.66	8	8	211.23	10.35	6	6	23.95	9.30	6	7	57.7
P02333 Relating type Lytoskeletar to OS=Mus musculus GN=Ext Strength Streng	39.26	11.57	1	1	32.47	11.57	1	1	68.48	11.57	1	1	13.3
P21460 Cystatin-C OS=Mus musculus GN=Cst3 PE=2 SV=2 - [CYTC_MOUSE]	55.20	11.07	-		56.17	11.57	-	-	46.65	11.43	1	1	15.5
P26645 Myristoylated alanine-rich C-kinase substrate OS=Mus musculus GN=Marcks PE=1 SV=2 - [MARCS_MOUSE]	57.36	5.50	1	1	56.58	10.36	2	2	65.57	5.50	1	1	29.6
Q02257 Junction plakoglobin OS=Mus musculus GN=Jup PE=1 SV=3 - [PLAK_MOUSE]	20.22	3.36	2	2	13.46	1.61	1	1	81.78	6.44	4	4	81.7
P16014 Secretogranin-1 OS=Mus musculus GN=Chgb PE=1 SV=2 - [SCG1_MOUSE]	58.83	4.43	2	2	73.81	4.43	2	2	60.54	9.60	5	5	77.9
P60041 Somatostatin OS=Mus musculus GN=Sst PE=3 SV=1 - [SMS_MOUSE]	25.94	9.48	1	1	59.83	9.48	1	1	55.93	9.48	1	1	12.7
Q3TTY5 Keratin, type II cytoskeletal 2 epidermal OS=Mus musculus GN=Krt2 PE=1 SV=1 - [K22E_MOUSE]	217.27	8.63	9	10	191.84	8.49	8	9	232.60	7.36	7	9	70.9
P62264 40S ribosomal protein S14 OS=Mus musculus GN=Rps14 PE=2 SV=3 - [RS14_MOUSE] P54227 Stathmin OS=Mus musculus GN=Stmn1 PE=1 SV=2 - [STMN1_MOUSE]	27.87	8.05	1	1					16.41	8.61	1	1	16.3
P94227 Stathmin OS=Mus musculus GN=Stmn1 PE=1 SV=2 - [STMN1_MOUSE] P0C056 Histone H2A.Z OS=Mus musculus GN=H2afz PE=1 SV=2 - [H2AZ_MOUSE]	26.65	7.81	1	1	24.52	7.81	1	1	16.76	7.81	1	1	17.3
Q8VED5 Keratin, type II cytoskeletal 79 OS=Mus musculus GN=Krt79 PE=2 SV=2 - [K2C79_MOUSE]	183.40	5.46	4	6	150.81	7.72	5	6	181.23	7.72	5	6	57.5
Q6NXH9 Keratin, type II cytoskeletal 73 OS=Mus musculus GN=Krt73 PE=1 SV=1 - [K2C73_MOUSE]	146.52	7.61	6	7	151.85	5.38	4	4	142.62	5.94	4	4	58.9
P35564 Calnexin OS=Mus musculus GN=Canx PE=1 SV=1 - [CALX_MOUSE]	27.32	3.38	1	1	44.87	2.03	1	2	96.47	6.94	3	4	67.2
P28667 MARCKS-related protein OS=Mus musculus GN=Marcksl1 PE=1 SV=2 - [MRP_MOUSE]	25.84	6.50	1	1		0.00			24.36	6.50	1	1	20.2
P01325 Insulin-1 OS=Mus musculus GN=Ins1 PE=1 SV=1 - [INS1_MOUSE]	25.20	6.48	1	1		0.00			17.43	6.48	1	1	12.2
P10923 Osteopontin OS=Mus musculus GN=Spp1 PE=1 SV=1 - [OSTP_MOUSE]				9					21.66	6.46	1	1	32.4
P04104 Keratin, type II cytoskeletal 1 OS=Mus musculus GN=Krt1 PE=1 SV=4 - [K2C1_MOUSE]	232.85 87.12	6.44 5.95	6	9	225.51 67.68	6.44 5.95	6	8	298.85 54.36	6.44 5.95	6	8	65.6 20.9
P63101 [14-3-3 protein zeta/delta OS=Mus musculus GN=Ywhaz PE=1 SV=1 - [1433Z MOUSE]	07.12	0.00	1	1	07.00	0.00	1	1	17.36	5.95	1	1	20.9
P60710 Actin, cytoplasmic 1 OS=Mus musculus GN=Actb PE=1 SV=1 - [ACTB_MOUSE]	66.20	5.60	2	2	38.90	2.93	1	1	37.85	5.60	2	2	41.7
Versity in the second secon	34.69	5.13	1	1	43.73	5.56	2	2	64.60	5.56	2	4	26.3
Q61171 Peroxiredoxin-2 OS=Mus musculus GN=Prdx2 PE=1 SV=3 - [PRDX2_MOUSE]	23.43	5.56	1	1	38.31	5.56	1	1		0.00			21.8
Q80854 Myelin expression factor 2 OS=Mus musculus GN=Myef2 PE=1 SV=1 - [MYEF2_MOUSE]	75.78	3.05	1	1	32.68	3.05	1	1	54.23	5.25	2	2	63.3
Q03517 Secretogranin-2 OS=Mus musculus GN=Scg2 PE=1 SV=1 - [SCG2_MOUSE]	75.24	3.08	2	2	56.70	3.57	2	2	88.90	3.08	2	2	70.6
Q9R1P4 Proteasome subunit alpha type-1 OS=Mus musculus GN=Psma1 PE=1 SV=1 - [PSA1_MOUSE]	25.24	4.94	1	1	51.34	4.94	1	1	19.27	4.94	1	1	29.5
Q62446 Peptidyl-prolyl cis-trans isomerase FKBP3 OS=Mus musculus GN=FKbp3 PE=1 SV=2 - [FKBP3_MOUSE]	42.26 82.43	4.91 4.55	1 5	1 5	84.82	2.86	3	3	78.86	3.03	3	3	25.1 62.8
Q3UV17 Keratin, type II cytoskeletal 2 oral OS=Mus musculus GN=Krt76 PE=2 SV=1 - [K220_MOUSE] Q06335 Amyloid-like protein 2 OS=Mus musculus GN=ApIp2 PE=1 SV=4 - [APLP2_MOUSE]	63.72	4.53	2	2	40.66	4.53	2	2	63.34	4.53	2	2	80.4
P08226 Apolipoprotein E OS=Mus musculus GN=Apoe PE=1 SV=2 - [APOE_MOUSE]	03.72	1.55	-	-	10.00	1.55	-	-	27.76	4.50	1	1	35.8
P07724 Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3 - [ALBU_MOUSE]	359.87	4.44	2	13	399.12	2.30	1	10	477.36	4.44	2	12	68.6
Q9DAR7 m7GpppX diphosphatase OS=Mus musculus GN=Dcps PE=1 SV=1 - [DCPS_MOUSE]									27.03	4.44	1	1	39.0
P07356 Annexin A2 OS=Mus musculus GN=Anxa2 PE=1 SV=2 - [ANXA2_MOUSE]									40.15	4.13	1	1	38.7
Q6IME9 Keratin, type II cytoskeletal 72 OS=Mus musculus GN=Krt72 PE=3 SV=1 - [K2C72_MOUSE]	52.32	1.73	2	2	95.84	4.04	3	3	94.63	4.04	3	4	56.7
P11499 Heat shock protein HSP 90-beta OS=Mus musculus GN=Hsp90ab1 PE=1 SV=3 - [HS90B_MOUSE]	79.39	3.87	2	2	143.38	2.21	2	2	137.39	4.01	3	4	83.2
Q61147 Ceruloplasmin OS=Mus musculus GN=Cp PE=1 SV=2 - [CERU_MOUSE] P63017 Heat shock cognate 71 kDa protein OS=Mus musculus GN=Hsna8 PE=1 SV=1 - [HSP7C_MOUSE]	48.45	0.94	1	1	73.90 21.43	2.26	2	2	84.63 21.00	3.72	3	4	121.1 70.8
P63017 Heat shock cognate 71 kDa protein OS=Mus musculus GN=Hspa8 PE=1 SV=1 - [HSP7C_MOUSE] P49312 Heterogeneous nuclear ribonucleoprotein A1 OS=Mus musculus GN=Hnrnpa1 PE=1 SV=2 - [ROA1 MOUSE]	23.99	3.44	1	1	15.83	3.44	1	1	18.57	3.44	1	1	34.2
P12023 Amyloid beta A4 protein OS=Mus musculus GN=App PE=1 SV=3 - [A4_MOUSE]	42.57	1.56	1	2	32.30	1.56	1	1	86.87	3.25	2	2	86.7
Q99JF8 PC4 and SFRS1-interacting protein OS=Mus musculus GN=Psip1 PE=1 SV=1 - [PSIP1_MOUSE]					25.30	2.65	1	1	22.05	2.65	1	1	59.7
Q64299 Protein NOV homolog OS=Mus musculus GN=Nov PE=2 SV=1 - [NOV_MOUSE]									23.45	2.54	1	1	38.9
P16858 Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=Gapdh PE=1 SV=2 - [G3P_MOUSE]	17.28	2.40	1	1					19.81	2.40	1	1	35.8
P80560 Receptor-type tyrosine-protein phosphatase N2 OS=Mus musculus GN=Ptprn2 PE=1 SV=2 - [PTPR2_MOUSE]	16.65	2.10	1	1									111.4
Q9EPL2 Calsyntenin-1 OS=Mus musculus GN=Clstn1 PE=1 SV=1 - [CSTN1_MOUSE]	24.91	1.33	1	1	10.00	0.00			24.14	0.72	1	1	108.8
Q00PI9 Heterogeneous nuclear ribonucleoprotein U-like protein 2 OS=Mus musculus GN=Hnrnpul2 PE=1 SV=2 - [HNRL2_M E90557 Deemonlakin OS=Mus musculus GN=Dan PE=1 SV=1 - [DESP. MOLISE]	44.04	1.88 0.52	1	1	42.72	1.88	1	1	44.78 57.43	1.88	1	1	84.9 332.7
E9Q557 Desmoplakin OS=Mus musculus GN=Dsp PE=1 SV=1 - [DESP_MOUSE] Q03157 Amyloid-like protein 1 OS=Mus musculus GN=ApIp1 PE=1 SV=1 - [APLP1_MOUSE]	42.65	1.84	1	1	42.95	1.84	1	1	42.41	1.35	5	1	332.7
P20152 Vimentin OS=Mus musculus GN=Vim PE=1 SV=3 - [VIME_MOUSE]	33.30	1.04	1		76.75	0.00		1	41.35	1.04	1	1	53.7
Q61316 Heat shock 70 kDa protein 4 OS=Mus musculus GN=Hspa4 PE=1 SV=1 - [HSP74 MOUSE]									19.95	1.66	1	1	94.1
P35396 Peroxisome proliferator-activated receptor delta OS=Mus musculus GN=Ppard PE=2 SV=1 - [PPARD_MOUSE]	27.94	1.59	1	1	35.81	1.59	1	1	24.81	1.59	1	1	49.7
Q99MR6 Serrate RNA effector molecule homolog OS=Mus musculus GN=Srrt PE=1 SV=1 - [SRRT_MOUSE]	40.37	1.49	1	1	34.23	1.49	1	1	25.83	1.49	1	1	100.4
P28862 Stromelysin-1 OS=Mus musculus GN=Mmp3 PE=2 SV=2 - [MMP3_MOUSE]	29.67	1.47	1	1	33.24	1.47	1	1	44.91	1.47	1	1	53.8
P12960 Contactin-1 OS=Mus musculus GN=Cntn1 PE=1 SV=1 - [CNTN1_MOUSE]									26.63	1.37	1	1	113.3
P38647 Stress-70 protein, mitochondrial OS=Mus musculus GN=Hspa9 PE=1 SV=3 - [GRP75_MOUSE]	23.59	1.33	1	1		0.00							73.4
P13595 Neural cell adhesion molecule 1 OS=Mus musculus GN=Ncam1 PE=1 SV=3 - [NCAM1_MOUSE]		0.00		_	41.79	1.26	1	1	38.49 21.41	1.26	1	1	119.4
Q61495 Desmoglein-1-alpha OS=Mus musculus GN=Dsg1a PE=2 SV=2 - [DSG1A_MOUSE] Q8BPM0 Disheveled-associated activator of morphogenesis 1 OS=Mus musculus GN=Daam1 PE=1 SV=4 - [DAAM1_MOUSE]		0.00							21.41 16.78	1.23	1	1	114.5
Q8BPM0 Disheveled-associated activator of morphogenesis 1 OS=Mus musculus GN=Daam1 PE=1 SV=4 - [DAAM1_MOUSE] O70228 Probable phospholipid-transporting ATPase IIA OS=Mus musculus GN=Atp9a PE=2 SV=3 - [ATP9A_MOUSE]						0.00			16./8	1.11	1	1	123.3
Probable prospholipid-transporting ATPase IIA US=Mus musculus GN=Atp9a PE=2 SV=3 - [ATP9A_MUUSE] P97350 Plakophilin-1 OS=Mus musculus GN=Pkp1 PE=2 SV=1 - [PKP1_MOUSE]	0.00	0.96	1	1	-	0.00			0.00	1.05	1	1	80.8
	0.00	0.50			10.00	0.86	1	1	25.55	0.04		1	126.5
999P72 Reticulor-4 OS=Mus musculus GN=Rh4 PE=1 SV=2 - [RTN4_MOUSE]					16.52					0.86			
Q99P72 Reticulon-4 OS=Mus musculus GN=Rtn4 PE=1 SV=2 - [RTN4_MOUSE]					16.52	0.86	1	1	34.12	0.52	1	1	254.2
					16.52	0.86	1	1			1 1	1 1	

Main Figures





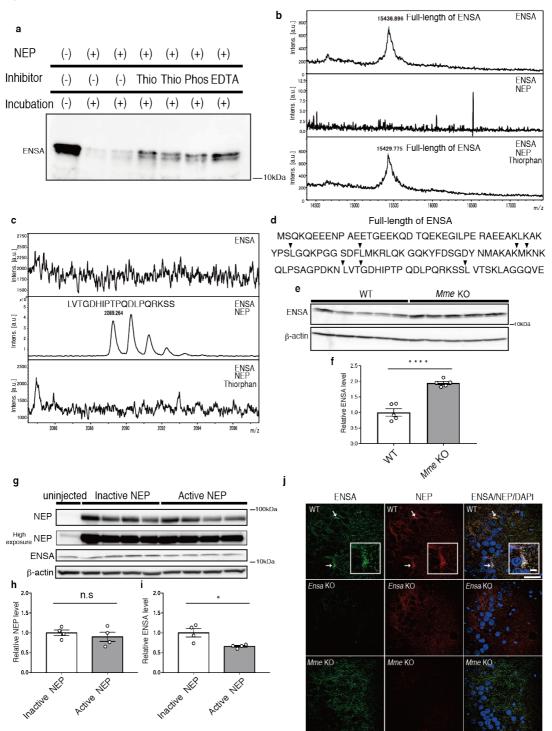
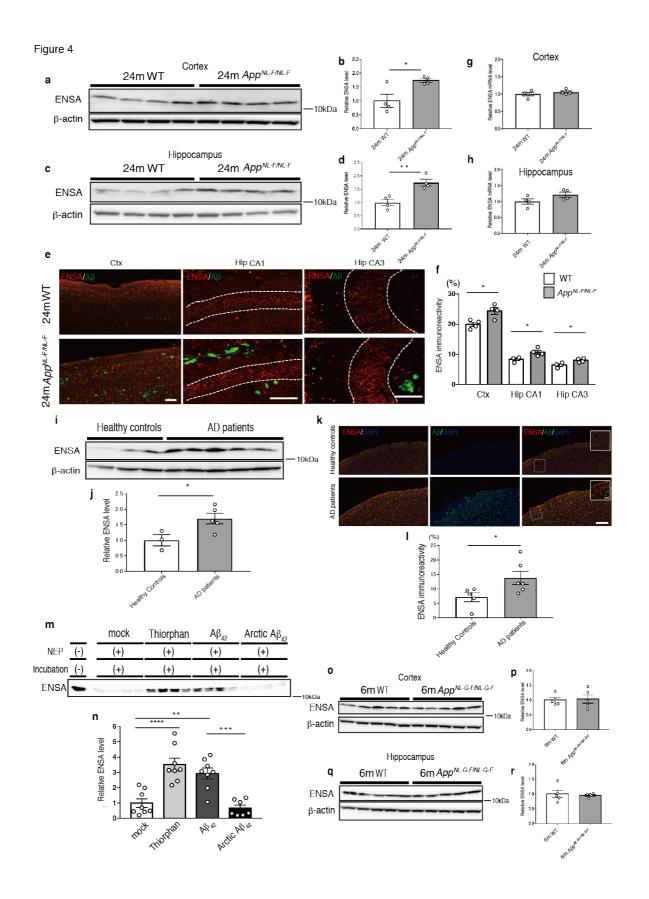
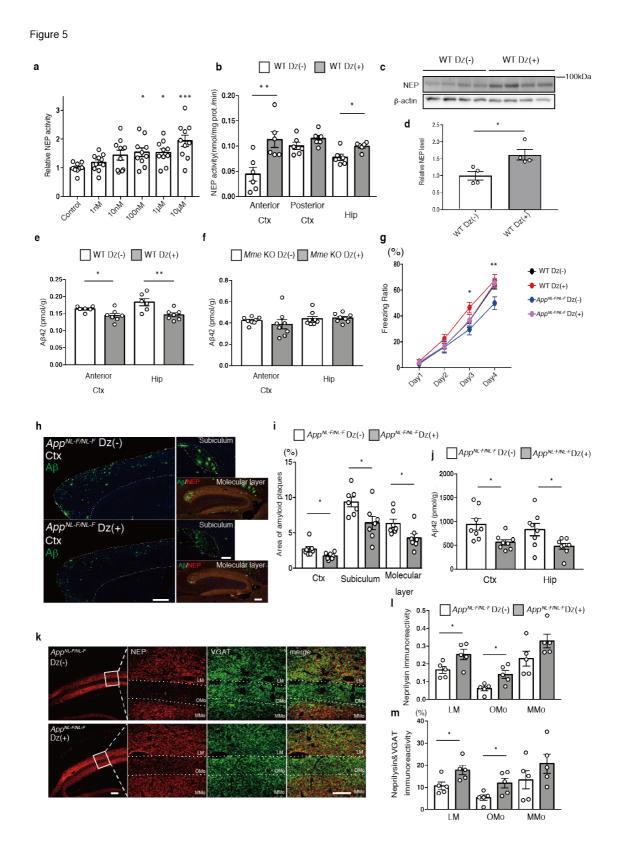


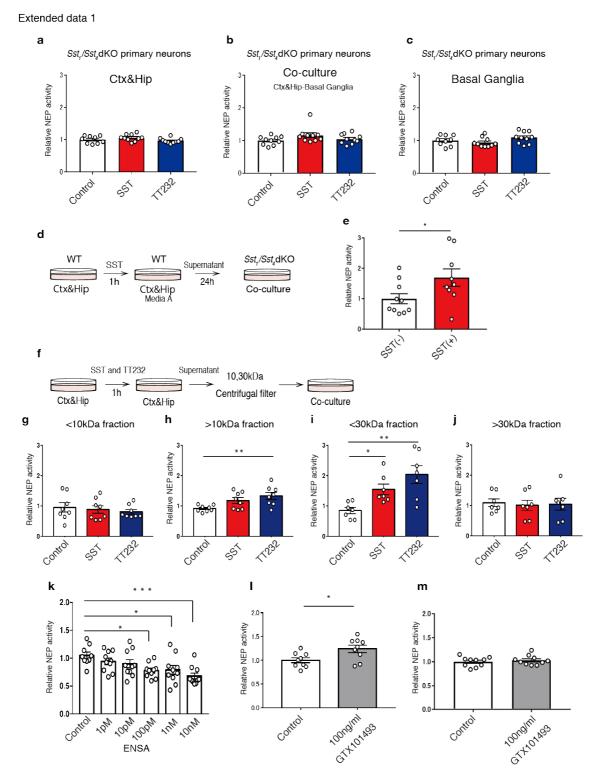
Figure 3





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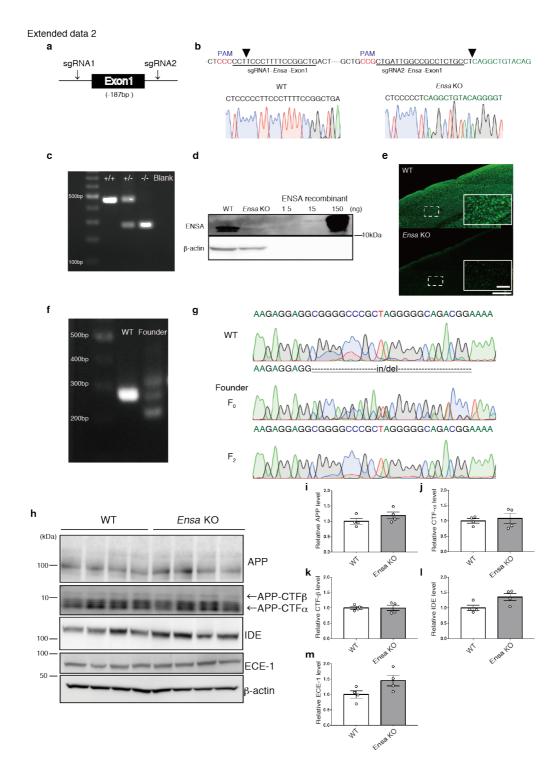
Supplemental data (Extended data)





activity after treatment of primary neurons derived from Sst_l/Sst_4 dKO mice with 1 μ M SST or TT232 for 24 hours. Cortical/hippocampal (Ctx&Hip) neurons (n = 9-10 wells per treatment), cocultured neurons (n = 10 wells per treatment), and basal ganglia neurons (n = 9-10 wells per treatment) were used. d, e, NEP activity of co-cultured neurons from Sst₁/Sst₄ dKO mice after replacement of the culture medium with conditioned media derived from SST-treated Ctx&Hip neurons from WT mice (n = 9-10 for each group). f-j, NEP activity of co-cultured neurons after replacement of the culture medium with separated conditioned media from Ctx&Hip neurons treated with SST or TT232. 10 and 30 kDa centrifugal filters were used for the separation (n = 7-8 for each group). k, NEP activity of co-cultured neurons derived from Sst₁/Sst₄ dKO mice after treatment with different doses of recombinant ENSA protein (n = 9-10 for each group). I, NEP activity of co-cultured neurons after neutralizing ENSA with 100 ng/ml of ENSA-specific antibody (GTX101493) (n = 9 for each group). g, NEP activity of co-cultured neurons derived from Ensa KO mice after neutralizing ENSA with 100 ng/ml ENSA-specific antibody (GTX101393) (n = 10 for each group). Information concerning *Ensa* KO mice is given in Extended Data Fig.2. In h, i, k, the data represent the mean ±SEM. *P<0.05, **P<0.01, ***P<0.001 (one-way ANOVA with Dunnett's post-hoc test). In e, l, the data represent the mean

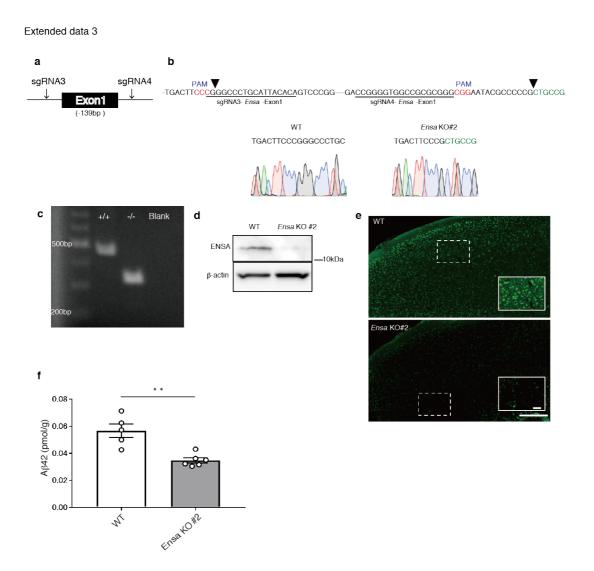
±SEM. **P*<0.05 (Student's *t*-test).



Extended data Fig.2 Generation of ENSA-deficient mouse using CRISPR/Cas9. a, Schematic

image for CRISPR/Cas9-mediated ENSA deficiency. b, Sanger sequence chromatograms near

exon 1 of *Ensa* gene in WT and *Ensa* KO mice. Arrowheads show Cas9 cleavage sites. **c**, PCRbased genotyping results of WT, heterozygous and homozygous *Ensa* KO mice. Genotyping was performed using mouse tail genome. **d**, Immunoblotting of ENSA in WT and *Ensa* KO mice. **e**, Immunostaining of ENSA in WT and *Ensa* KO mice. ENSA immunoreactivity is absent in *Ensa* KO mice. Scale bar = 500 μ m. Inset Scale bar = 50 μ m. **f**, PCR-based genotyping results of offtarget sites in WT and founder *Ensa* KO mice. Genotyping was performed using mouse tail genome. **g**, Sanger sequence chromatograms of off-target sites in WT, founder mouse and F2 *Ensa* KO mouse. **h**, Immunoblotting of APP, CTFs, IDE and ECE-1 in 3-month-old WT and *Ensa* KO mice. **i-m**, Values indicated in graphs show band intensities for APP, CTFs, IDE and ECE-1 normalized to that of β-actin (n = 4 for each group). Results are expressed as the mean ±SEM.



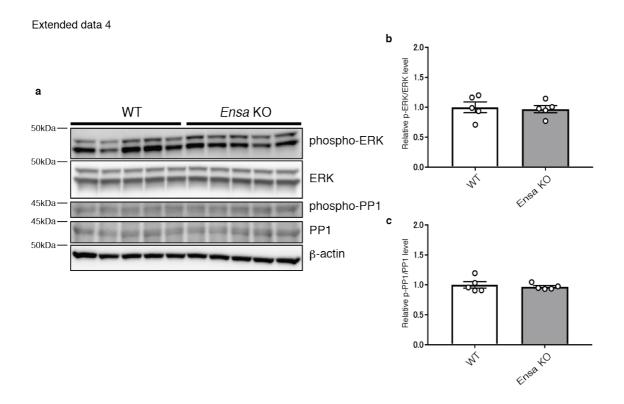
Extended data Fig.3 Generation of 2nd line ENSA-deficient mouse using CRISPR/Cas9. a,

Schematic image for CRISPR/Cas9-mediated ENSA deficiency. **b**, Sanger sequence chromatograms near exon 1 of *Ensa* gene in WT and *Ensa* KO #2 mice. Arrowheads show cleavage sites by Cas9. **c**, PCR-based genotyping results of WT and *Ensa* KO #2 mice. Genotyping was performed using mouse tail genome. **d**, Immunoblotting of ENSA in WT and *Ensa* KO #2 mice. **e**, Immunostaining of ENSA in WT and *Ensa* KO #2 mice. ENSA

immunoreactivity was absent in *Ensa* KO #2 mice. Scale bar = 500 μ m. Inset scale bar = 50 μ m.

f, AB₄₂ ELISA of hippocampi from 3-month-old WT and *Ensa* KO #2 mice (WT: n = 5, *Ensa* KO

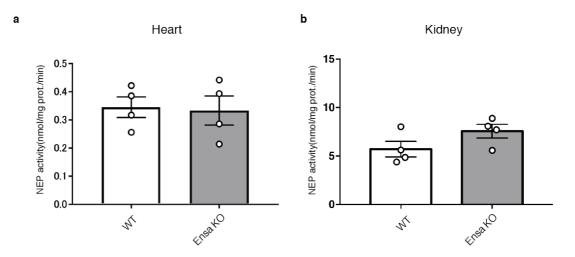
#2: n = 6). Results are expressed as the mean \pm SEM. ***P*<0.01 (Student's *t*-test).



Extended data Fig.4 Mechanism for translocalization of NEP in *Ensa* KO mice. a-c, Immunoblotting of phospho-ERK, ERK, phospho-PP1 and PP1 in hippocampi of 3-month-old ENSA KO mice. Values indicated in **b** show phospho-ERK band intensities normalized to those of ERK and in **c**, phospho-PP1 band intensity normalized to that of PP1 (n = 5 for each group).

Results are expressed as the mean \pm SEM.



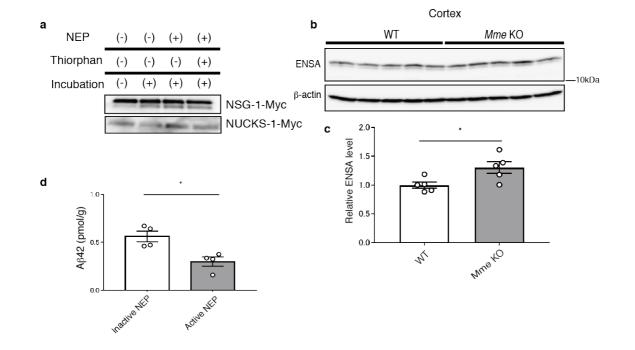


Extended data Fig.5 Cardiac and renal NEP activity in Ensa KO mice. a, NEP activity in

membrane fractions from cardiac tissue of 3-month-old WT and *Ensa* KO mice (n = 4 for each group). **b**, NEP activity in the membrane fractions from kidney tissue of 3-month-old WT and

Ensa KO mice (n = 4 for each group). Results are expressed as the mean \pm SEM.

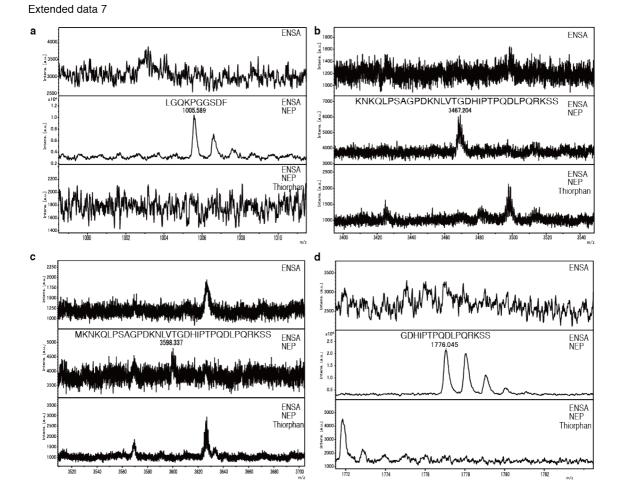
Extended data 6



Extended data Fig.6 Identification of ENSA as a substrate for NEP. a, Immunoblotting of

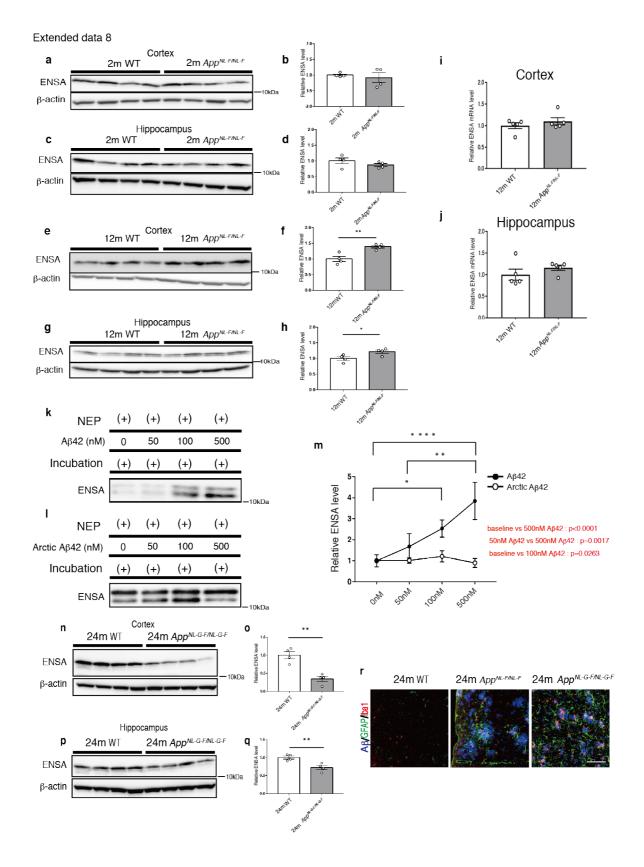
NSG-1 or NUCKS-1 incubated with or without NEP and thiorphan for 24 hours at 37°C. The specific band is detected by Myc-tag antibody. **b**, Immunoblotting of ENSA in cortices from 6-month-old WT and *Mme* KO mice. Values indicated in the graph show the intensity of ENSA bands normalized to that of β -actin (n = 5 for each group). **c**, A β_{42} ELISA of Tris-HCl-buffered saline-soluble fractions containing 1% Triton-X from hippocampi of WT mice after overexpression of active or inactive mutant NEP by the SFV gene expression system (n = 4 for each group). Results are expressed as the mean ±SEM. **P*<0.05 (Student's *t*-test).

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Extended data Fig.7 Specific peaks of ENSA cleaved by NEP. a-d, MALDI-TOF analyses

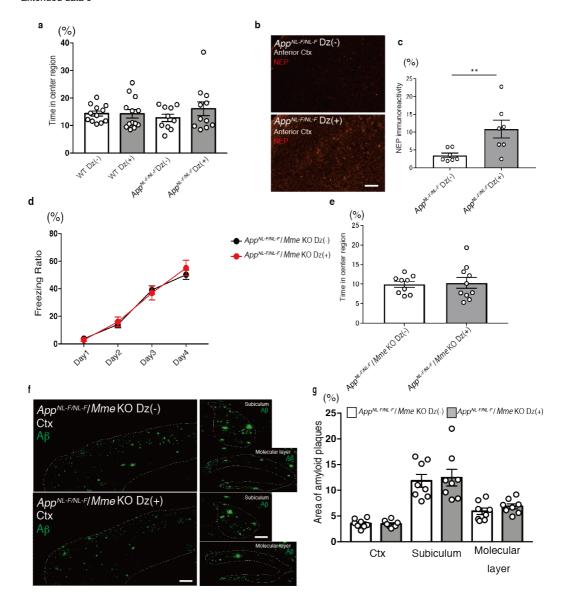
showing specific peaks of cleaved ENSA after incubation in the presence or absence of NEP and thiorphan for 24 hours at 37°C. LC-MS/MS analysis was used to determine specific amino acid sequences (Supplemental Table 3).



Extended data Fig.8 ENSA levels in App^{NL-F} and App^{NL-G-F} mice. a-d, Immunoblots of ENSA

in cortices and hippocampi of 2-month-old WT and App^{NL-F} mice. Values indicated in the graph show the intensities of ENSA bands normalized to that of β -actin (n = 4 for each group). e-h, Immunoblots of ENSA in cortices and hippocampi from 12-month-old WT and App^{NL-F} mice. Values indicated in the graphs show the intensities of ENSA bands normalized to that of β-actin (n = 4 for each group). i,j, Semi-quantification of ENSA mRNA levels in cortices and hippocampi of WT and App^{NL-F} mice at 12 months. Values indicated in the graphs show ENSA levels normalized to that of G3PDH (n = 4 for each group). k-m, Immunoblots of ENSA incubated with NEP and specified levels of $A\beta_{42}$ or Arctic $A\beta_{42}$ (n = 4 for each group). n-q, Immunoblots of ENSA in cortices and hippocampi of 24-month-old WT and App^{NL-G-F} mice. Values indicated in the graph show ENSA band intensities normalized to that of β -actin (n = 4 for each group). r, Immunostaining of Aβ, GFAP and Iba1 in 24-month-old WT, App^{NL-F} and App^{NL-F} ^{G-F} mice. Scale bar = 100 μ m. In **f**, **h**, **o**, **q**, results are expressed as the mean ±SEM. *P<0.05, ** $P \le 0.01$, (Student's *t*-test). In **m**, results are expressed as the mean \pm SEM. * $P \le 0.05$, ** $P \le 0.01$,

****P<0.0001 (two-way ANOVA with Turkey's multiple comparison test).



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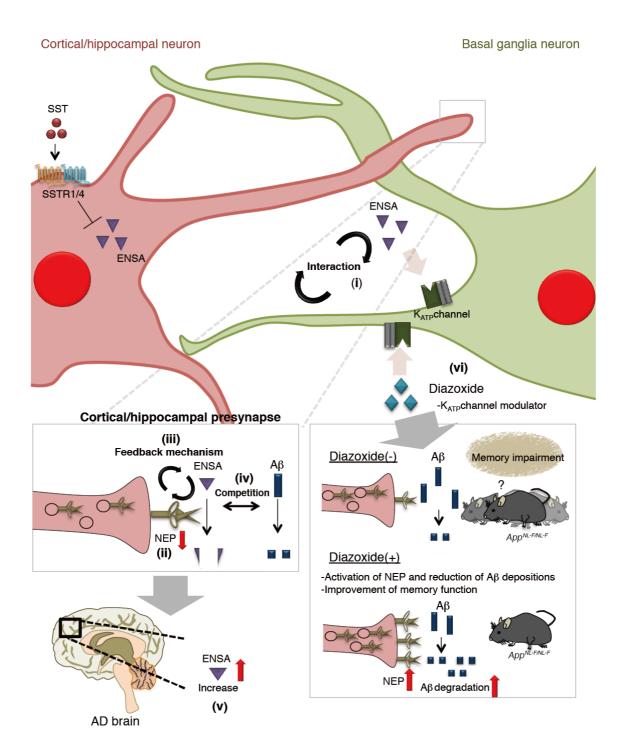
Extended data Fig.9 Improvement of Aß pathology in AD model mouse via enhancement of

NEP activity by diazoxide treatment. a, Statistical analysis of open field test to measure time in central region. 18-month-old WT and App^{NL-F} mice were treated with or without Dz for 3 months (WT Dz (-): n = 12, WT Dz (+): n = 13, App^{NL-F} Dz (-): n = 10, App^{NL-F} Dz (+): n = 11).

b,c, Immunostaining of NEP in cortices of 18-month-old WT and App^{NL-F} mice treated with or without Dz for 3 months (n = 7 for each group). Scale bar = 500 µm. **d,** Freezing ratio of 15-month-old App^{NL-F}/Mme KO mice treated with or without Dz for 3 months $(App^{NL-F}/Mme$ KO Dz (-): n = 9, App^{NL-F}/Mme KO Dz (+): n = 10). **e,** Statistical analysis of open field test to measure time in central region of maze. 15-month-old App^{NL-F}/Mme KO Dz (-): n = 9, App^{NL-F}/Mme KO Dz (-): n = 9, App^{NL-F}/Mme KO Dz (+): n = 10). **f,** Immunostaining of A β (Green) in cortex, subiculum and molecular layer of 15-month-old App^{NL-F}/Mme KO mice with or without Dz for 3 months. Scale bar in cortical image = 500 µm and in hippocampal image = 200µm. **g,** Statistical analysis of amyloid plaque area in 15-month old App^{NL-F}/Mme KO treated with or without Dz for 3 months (n = 8 for each group). Results expressed as the mean ±SEM. **P<0.01 (Mann-Whitney test).

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Extended data Fig.10 Schematic of key findings of this study.

SST-induced NEP activity is regulated by the interaction between cortical/hippocampal and basal ganglia neurons *in vitro* (i). ENSA is a negative NEP regulator (ii). NEP activity may be regulated by a substrate-dependent feedback mechanism (iii). A β and ENSA compete with each other in NEP-mediated degradation (iv). ENSA is elevated in aged App^{NL-F} mice and in patients with AD(v). K_{ATP} channel modulator diazoxide increases NEP activity and decreases amyloid

deposition in *App^{NL-F}* mice resulting in improvement of memory function (vi).