Supplementary Information Titles

Nature Medicine	
Article title	A noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of β-secretase expression
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Supplementary methods	Materials and methods
Supplementary Figure-S1	Quantitative expression analysis of *Bace1* and *Bace1-as* in four different regions of the mouse brain by RT-PCR
Supplementary Figure-S2	*BACE1* and *BACE1-AS* expression in differentiated SH-SY5Y cells
Supplementary Figure-S3	BACE2 mRNA concentrations were not affected by BACE1-AS siRNA
Supplementary Figure-S4	Enzyme Fragment Complementation (EFC) technology
Supplementary Figure-S5	siRNA and shRNA mediated depletion of *BACE1-AS* causes BACE1 protein down-regulation
Supplementary Figure-S6	*BACE1-AS* mRNA is elevated in AD individuals
Additional data file-1	RACE sequencing results of the human *BACE1-AS*
Additional data file-2	RACE sequencing results of the mouse *Bace1-as*
Supplementary Table-S1	list of primer, probe and siRNA sequences

Methods (online supplementary)

Rapid Amplification of cDNA Ends (RACE): Sequence information for human *BACE1* (NM_012104), mouse *Bace1* (NM_011792), and potential human (CB960709) and mouse (AK074428 and AK078885) *Bace1* natural antisense transcripts were retrieved from the UCSC Genome Bioinformatics web site. Using RACE ready cDNA (Ambion) from human and mouse brain we amplified the 5' and 3' ends of *BACE1-AS* by nested PCR with gene specific and kit primers. We excised the 3' and 5' PCR products of both mouse and human from the gel and cloned them into the T-Easy vector (Promega). We sequenced twenty positive colonies from each series.

Real-Time PCR (RT-PCR): We carried out RT-PCR with the GeneAmp 7,900 machine (Applied Biosystems). The PCR reactions contained 20–40 ng cDNA, Universal Mastermix, 300 nM of forward and reverse primers, and 200 nM of probe in a final reaction volume of 15 µl (primers and probe sequences are listed in **Supplementary Table S1** online). We designed the primers and probe using FileBuilder software (Applied Biosystems). They were strand-specific for sense-antisense pairs and the sense probes covered exon boundaries to eliminate the chance of genomic DNA amplification. The PCR conditions for all genes were as follows: 50 ºC for 2 min then 95 ºC for 10 min then 40 cycles of 95 ºC for 15 s and 60 ºC for 1 min. The results are based on cycle threshold (Ct) values. We calculated differences between the Ct values for experimental and reference genes (18s RNA) as ΔΔCt and graphed as a percent of each RNA to the calibrator sample. We assessed the relative expression of *BACE1* and *BACE1-AS* RNA

transcripts in several human and mouse cell lines, by real time PCR (RT-PCR). We measured the expression of *Bace1* and *Bace1-as* transcripts in various regions of the mouse brain and liver in wild-type mice (n = 3) by RT-PCR. Both transcripts are co-expressed in various tissues and cell lines tested, and the expression levels of *BACE1* mRNA expression levels were between 25% and 75% greater than *BACE1-AS* transcript

RNA extraction and RT-PCR of the mouse brain samples: We euthanized mice after 14 days and removed the brains. We excised five tissues from each mouse for RNA quantitative measurement; dorsal hippocampus, ventral hippocampus, cortex, dorsal striatum and cerebellum. We extracted RNA after homogenization in Trizol reagent (Invitrogen) according to the manufacturer's protocol. We passed the extracted RNA samples through Qiagen RNeasy columns and we subjected those RNA samples to on-column DNAse treatment for removal of DNA contamination. We used 800 ng of each sample for the first strand cDNA synthesis and carried out RT-PCR measurements as described above. We plotted the percentile changes in RNA levels, for individual tissues compared to control mice, in each graph.

Cell culture and transfection: We cultured SH-SY5Y cells in a mixture of MEM and F12 plus 10% FBS, 1% NEAA, 1% L-glutamate and 1% sodium bicarbonate. We transfected cells in logarithmic growth, with 20 nM of siRNA using 0.2% Lipofectamine 2,000 according to manufacturer's instructions (Invitrogen). Cells were incubated for 48 h prior to further use.

For shRNA transfection, we purchased the short hairpin RNA vectors from OriGene technologies (OriGene) and transformed and transfected using their protocol. After transfection of shRNA, we kept HEK293T cells on Puromycin (2–10 μg/ml) for 2 weeks. After 3–4 weeks of cell selection via puromycin (2–10 μg/ml) treatment, we measured RNA transcripts by RT-PCR.

We cloned full sequences of human *BACE1-AS* into pcDNA 3.1 (–) vector (Invitrogen). We transfected HEK293T cells with *BACE1-AS* vector or empty vector served as a control, and selected by adding neomycin for two weeks. We also performed transfection of HEK293T cells with a vector containing *BACE1-AS* transcript. After transfection, total RNA was isolated and *BACE1* and *BACE1-AS* mRNA levels were measured by RT-PCR. The *BACE1-AS* transcript was increased as predicted; furthermore this overexpression of *BACE1-AS* also led to increase in *BACE1* mRNA and protein.

For induction of neuronal like differentiation, we exposed SH-SY5Y cells to 20 μM of retinoic acid (Sigma) for 2 weeks.

We cultured HEK-SW cells, which artificially overexpressing APP Swedish mutation, on D-MEM supplemented with 10% FBS and transfected with 20 nM of siRNA against either *BACE1*, *BACE1-AS* or control siRNA. We measured expression levels of *BACE1*, *BACE2* and *BACE1-AS* transcripts by RT-PCR. In a separate experiment and to examine the dose response curve we transfected these cells with a range of 100 pm to 20 nM *BACE1-AS* siRNA.

The 7PA2-CHO cells were previously shown to overproduce Aβ 1-42 dimers and oligomers. We cultured 7PA2-CHO cells and parental control CHO cells on D-MEM

supplemented with 10% FBS. Conditioned media from these cells or control parental CHO cells were collected and added to SH-SY5Y cells for 2 h after removal of the regular media.

We purchased human cortical neurons, HCN-1A, originating from the brain of an 18-month old female from ATCC (ATCC # CRL-10442). These cells were reported to be positive for a number of neuronal markers including neurofilament protein, neuron specific enolase (NSE) and gamma aminobutyric acid (GABA). We cultured cells in a D-MEM medium supplemented with 10% FBS.

We purchased human glial cells, M059K originated from the brain of a 33 years old male with malignant glioblastoma, from ATCC (ATCC # CRL-2365). We cultured these cells in a mixture of D-MEM and F12 plus 10% FBS, 1% NEAA, 2.5 mM L-glutamate, 15 mM HEPES, 0.5 mM sodium pyruvate, and 1% sodium bicarbonate. We measured expression levels of *APP*, *BACE1* and *BACE1-AS* transcripts by RT-PCR.

Induction of various cell stressors: We plated HEK-SW cells in 6-well plates and exposed them to the cell stressors for 12 h as follow: 1- H_2O_2; 50 µM, 2- hyperosmolar glucose; 25 mM (regular media contains 5.56 mM glucose), 3- Aβ 1-42; one ng /ml, 4- Aβ 1-40; one ng /ml, 5- hyperthermia; 42 ºC, 6- serum starvation; no serum containing regular media, 7- Staurosporine; 100 nM, 8- control untreated. We extracted RNA's and synthesized cDNA for RT-PCR. The results are based on cycle threshold (Ct) values. We calculated differences between the Ct values for experimental and reference genes (*β-Actin*) as ΔΔCt. In separate experiment, we treated SH-SY5Y cells with synthetic Aβ 1-

42 peptides (1 μM for 2 h) as stressor and monitored changes in cytoplasmic and nuclear *BACE1-AS* and other RNA transcripts by RT-PCR.

Northern blotting: We purchased the FirstChoice Northern human blot 1 from Applied Biosystems (Applied Biosystems). Premade blot were produced by RNA originated from human tissues of different subjects (see below, $n = 1$ per RNA). We blocked the membrane with Ultrahybrid (Ambion) for 6 h and hybridized it overnight with radiolabeled *BACE1* or *BACE1-AS* probes spanning most of the overlap region of the human *BACE1* and *BACE1-AS*. We made the probes by *in vitro* transcription of cloned *BACE1-AS* overlap region using T7 promoter and ^{32}P-labeled UTP nucleotide (PerkinElmer). We washed the membrane once with low stringency and twice with high stringency buffer, each for 10 min, and detected signals with a phosphor-imaging instrument. Total RNA were blotted on the membrane from left to right 1- Brain; 68 Y/O male, 2- Placenta; 27 Y/O female, 3- Skeletal muscle; 79 Y/O female, 4- Heart; 70 Y/O female, 5- Kidney; 60 Y/O male, 6- Pancreas; 45 Y/O male, 7- Liver; 64 Y/O male, 8- Lung; 46 Y/O male, 9- Spleen; 39 Y/O male, 10- Colon; 23 Y/O male. Single stranded RNA probes bounds to 5.9 kb *BACE1* and ~ 2kb *BACE1-AS* transcripts.

Northern data revealed expression of both transcripts in brain and a range of other tissues. While we readily detected *BACE1-AS* and *BACE1* not only in brain but also in pancreas, liver, lung and spleen, the expression levels of both transcripts were lower in colon, placenta and kidney. We observed that pancreas showed multiple splice variants of *BACE1*. These expression data demonstrate widespread co-expression of both transcripts in various human tissues.

Statistical analysis: We performed all experiments with 6–20 biological and 3–6 technical repeats. The data presented in graphs as a comparison with control-treated groups, after post-hoc test of treatment factor using main effect in two-way analysis of variance (ANOVA). We calculated the significance of each treatment as a p value and depicted in each graph, ($p < 0.05$) was considered significant.

Western blotting: We transfected HEK293T cells with 20 nM of *BACE1*, *BACE1-AS*, or control siRNA. Alternatively, we used shRNA stable cell lines expressing *BACE1* shRNA, *BACE1-AS* shRNA or control shRNA for Western blotting. We disrupted cells, 48 h post transfection, with 200 µl of Laemmli sample buffer (Biorad) containing 350 mM DTT. We extracted total protein from mouse brain tissues embedded in Trizol reagent (Invitrogen). We separated 20 µl of the lysate on a 10% SDS PAGE and transferred it to a nitrocellulose membrane overnight. We incubated the membrane with primary antibody for BACE1 (Abcam) and secondary antibody conjugated to HRP. After addition of HRP substrate, we detected the chemiluminescence signal with X-ray film. We stripped the same membrane and reused it for detection of β-Actin as a loading control.

ELISA and HTRF assay: We seeded HEK-SW cells, artificially overexpressing APP Swedish mutation, on 6-well plates and transfected with 20 nM of *BACE1* siRNA, *BACE1-AS* siRNA or control siRNA. We collected supernatant media from the HEK-SW cells, 48 h after transfection, for detection of Aβ 1-40, Aβ 1-42 and soluble APP

alpha (sAPPα). We lysed HEK-SW cells for detection of total APP. We purchased the ELISA kits for Aβ 1-40, Aβ 1-42 and APP from Invitrogen (Invitrogen) and we performed ELISA following supplier protocol. We subtracted average absorbance of three repeats at 450 nm from background and normalized it to negative control siRNA sample. We utilized a Time Resolved Fluorescence (HTRF) assay (Cisbio) for Aβ 1-42 detection. Two specific monoclonal antibodies against Aβ 1-42 were tagged to flurophores and upon binding to the Aβ peptide simultaneously, and based on distance between them, emission of the first one will excite the second. We performed HTRF reaction following manufacturer's protocol, using purified protein from APP–tg and wild-type mice, without any washing steps which allowed direct measurement of the peptide.

Ribonuclease Protection Assay (RPA): We treated RNA from SH-SY5Y cells with ribonuclease A+T which digests single stranded RNAs but not RNA duplexes. Following the RPA assay we used RT-PCR to detect *BACE1* and *BACE1-AS* employing two sets of primers and probes. We designed the first set of primers to target the overlapping region of the BACE1 sense and antisense transcripts and the second set to target non-overlapping region of these transcripts. We incubated RNA samples at 37 °C for 1 h before treatment with RNAse A + T cocktail (Ambion). After adding RNAse cocktail, we incubated samples 30 min at 37 °C, and treated with proteinase K. We extracted RNA afterward using RNeasy kit (QIAGEN). We treated RNAse minus control in the same way and we carried out cDNA synthesis and RT-PCR as mentioned

above, with two distinct primer and probe sets for overlapping and non-overlapping part of *BACE1* and *BACE1-AS* transcripts.

Stability and α-amanitin treatment: We plated HEK293T cells, *BACE1-AS* shRNA, control shRNA stable cell lines or *BACE1-AS* over expressing cells into 6-well plates. We treated cells 24 h later with 50 μg/ml of α-amanitin and harvested cells for RNA purification and RT-PCR at 6, 12, and 24 h post treatment. We taken three independent samples for each data point and all samples had untreated and untransfected matching samples for RNA purification and data analysis.

Supplementary Figure-S1

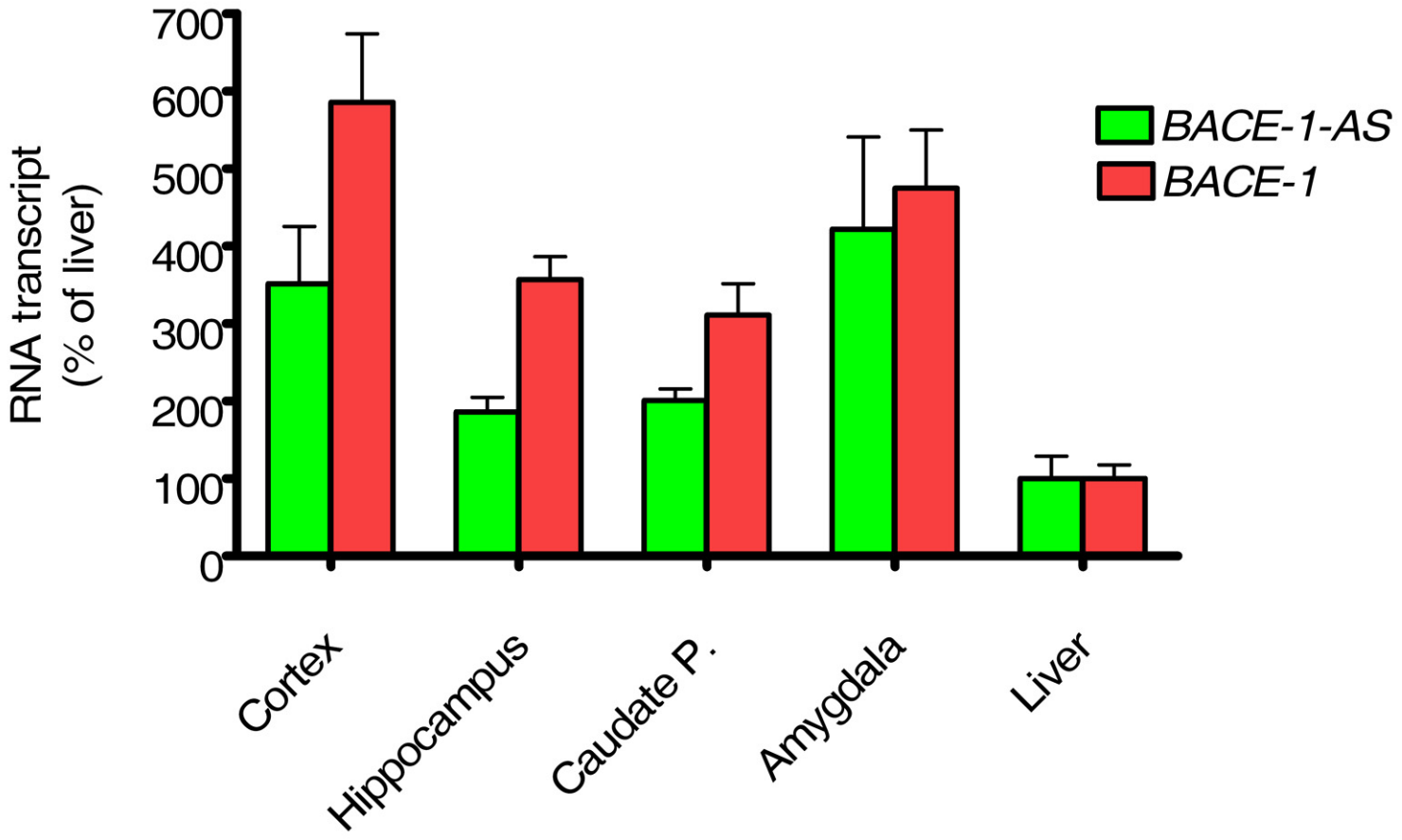


Figure S1. Quantitative expression analysis of *Bace1* and *Bace1-as* in four different regions of the mouse brain by RT-PCR: Three wild-type male mice were euthanized and tissues were excised for RT-PCR analysis. Both transcripts were abundant in all tissues tested. The expression in various region of the brain is normalized to the expression in liver.

Supplementary Figure-S2

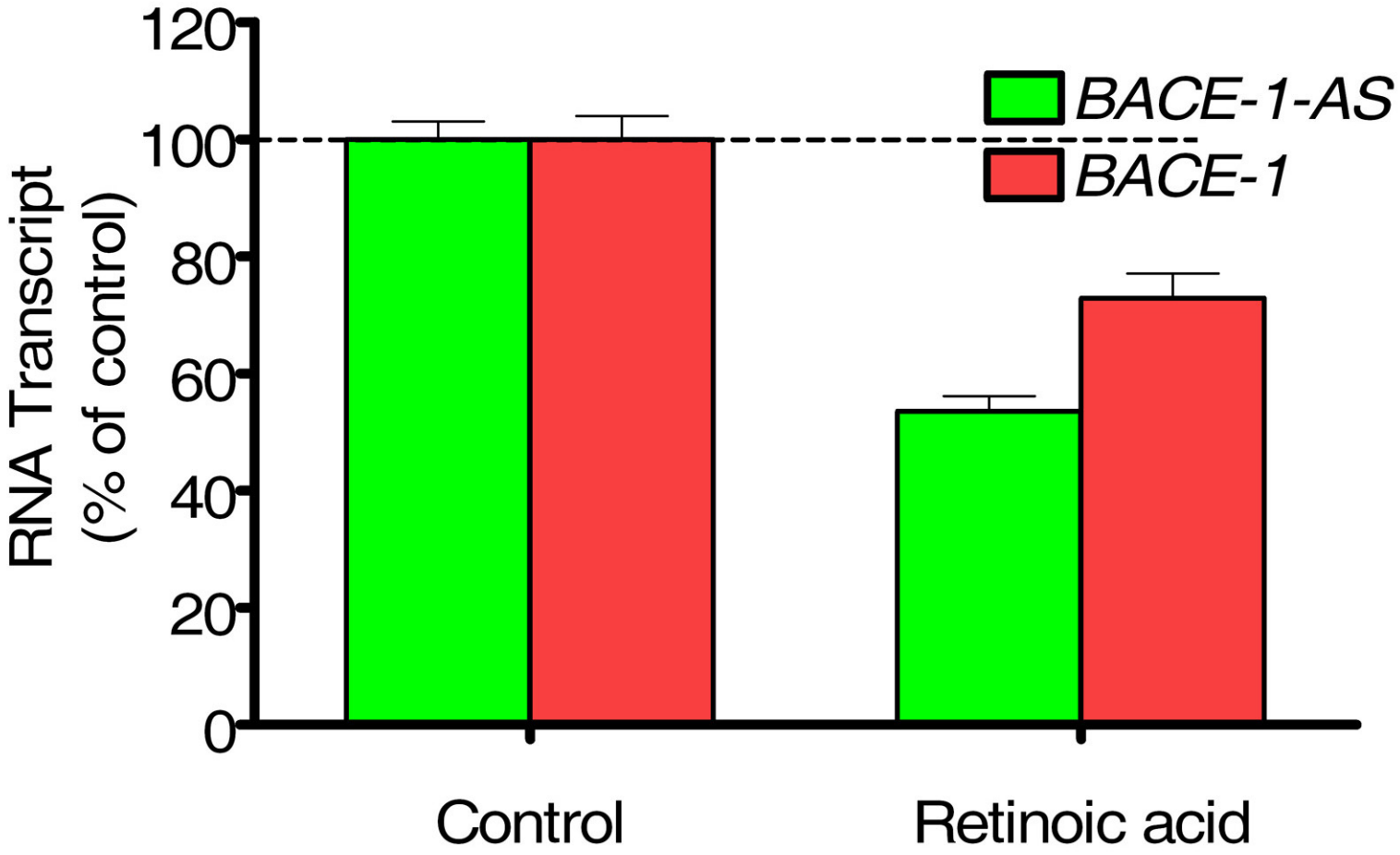


Figure S2. ***BACE1*** **and** ***BACE1-AS*** **expression in differentiated SH-SY5Y cells**: Induction of differentiation of SH-SY5Y cells with retinoic acid (20 µM) for two weeks resulted in a reduction of *BACE1-AS* transcript expression by about 50%, and also the *BACE1* transcript by about 20% (*P< 0.0001*).

Supplementary Figure-S3

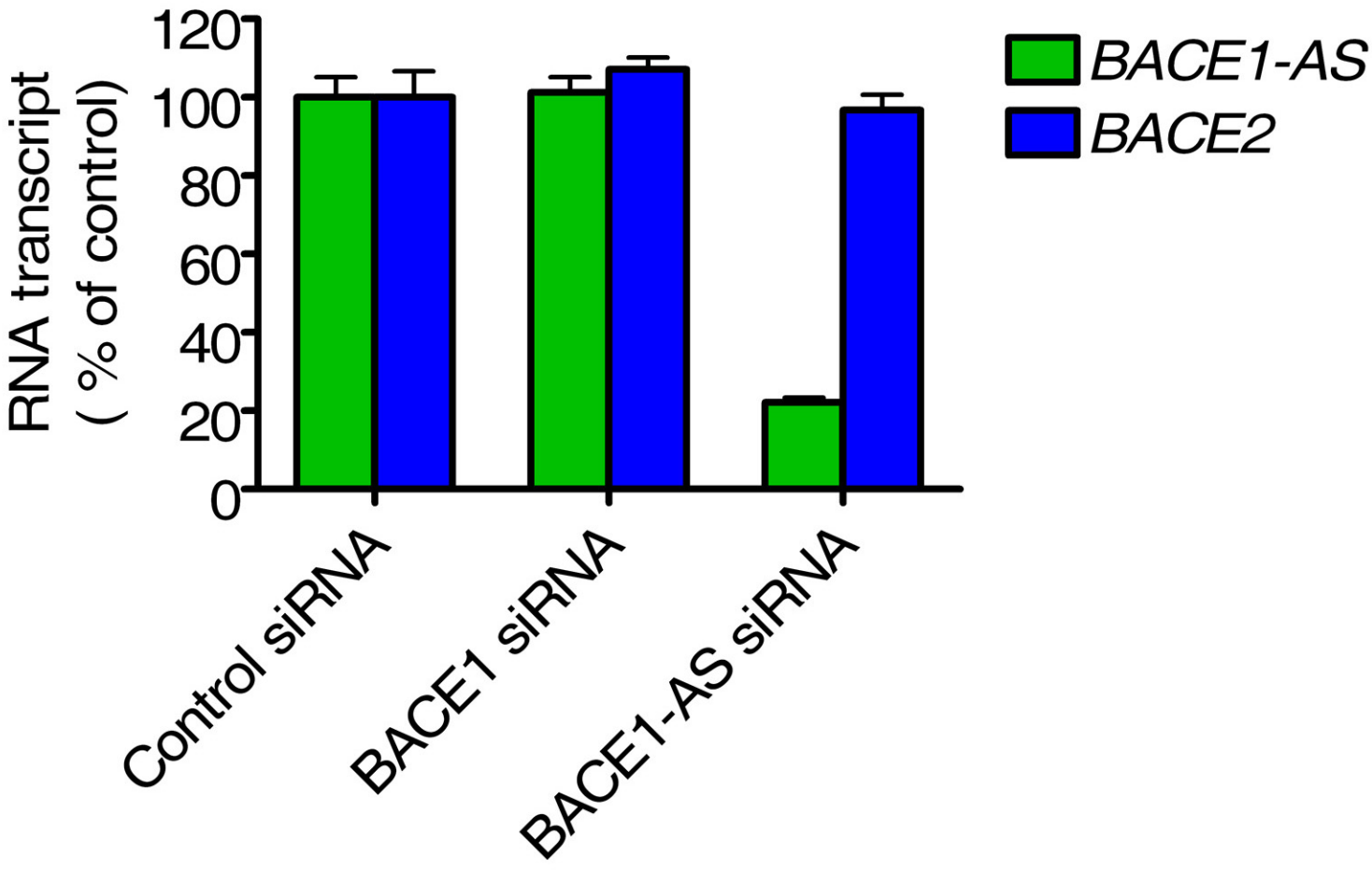
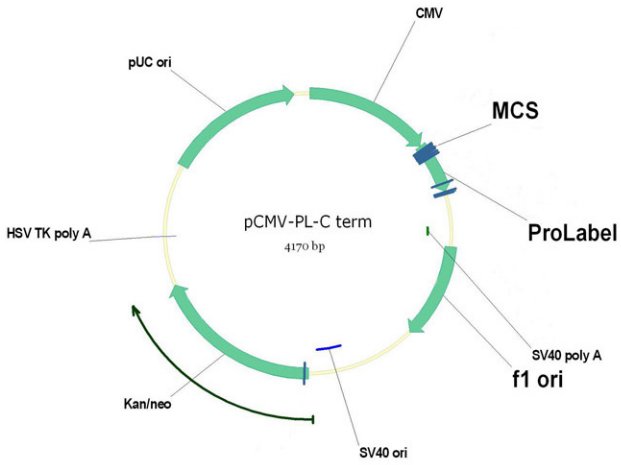


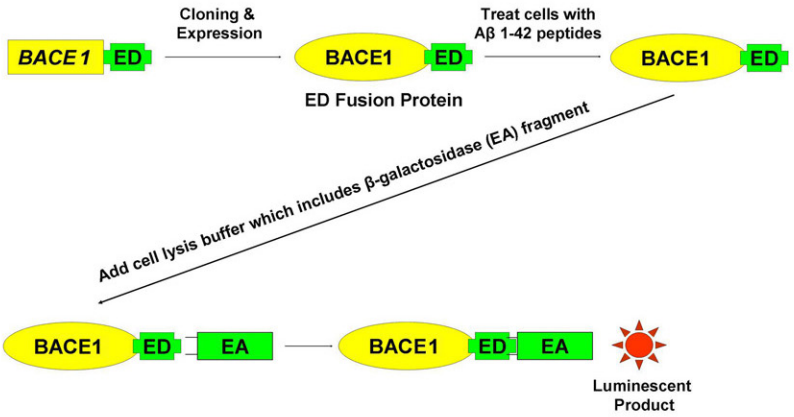
Figure S3. BACE2 mRNA is not affected by BACE1-AS siRNA: We transfected HEK-SW cells with 20nM of *BACE1-AS*, *BACE1* or control siRNA. After 48 h, we measured levels of *BACE1-AS* and *BACE2* RNA by RT-PCR. The concentrations of *BACE2* were not changed by siRNA treatment, supporting the specificity of the observed *BACE1* concordant regulation by *BACE1-AS*.

Supplementary Figure-S4

(**a**) ProLabel vector



(**b**) ED fusion construct and β-galactosidase complementation assay



Strength of luminescent signals is proportional to amount of protein

Figure S4. Enzyme Fragment Complementation (EFC) technology (DiscoveRx):

(a) ProLabel vector supplied by DiscoveRx is used to clone *BACE1* cDNA upstream of the ProLabel to produce BACE1 protein with the β-galactosidase enzyme donor (ED) fragment.

(b) We treated cells expressing BACE1 ED fusion protein with Aβ 1-42 peptides for 12 h. Thereafter, provided lysis buffer that includes the enzyme acceptor (EA) fragment of β-galactosidase is added to the cells, which leads to the activation of the β-galactosidase enzyme and hydrolysis of a substrate that produces a luminescent signal. The strength of this signal is measured to determine the change in BACE1 protein levels.

Supplementary Figure-S5

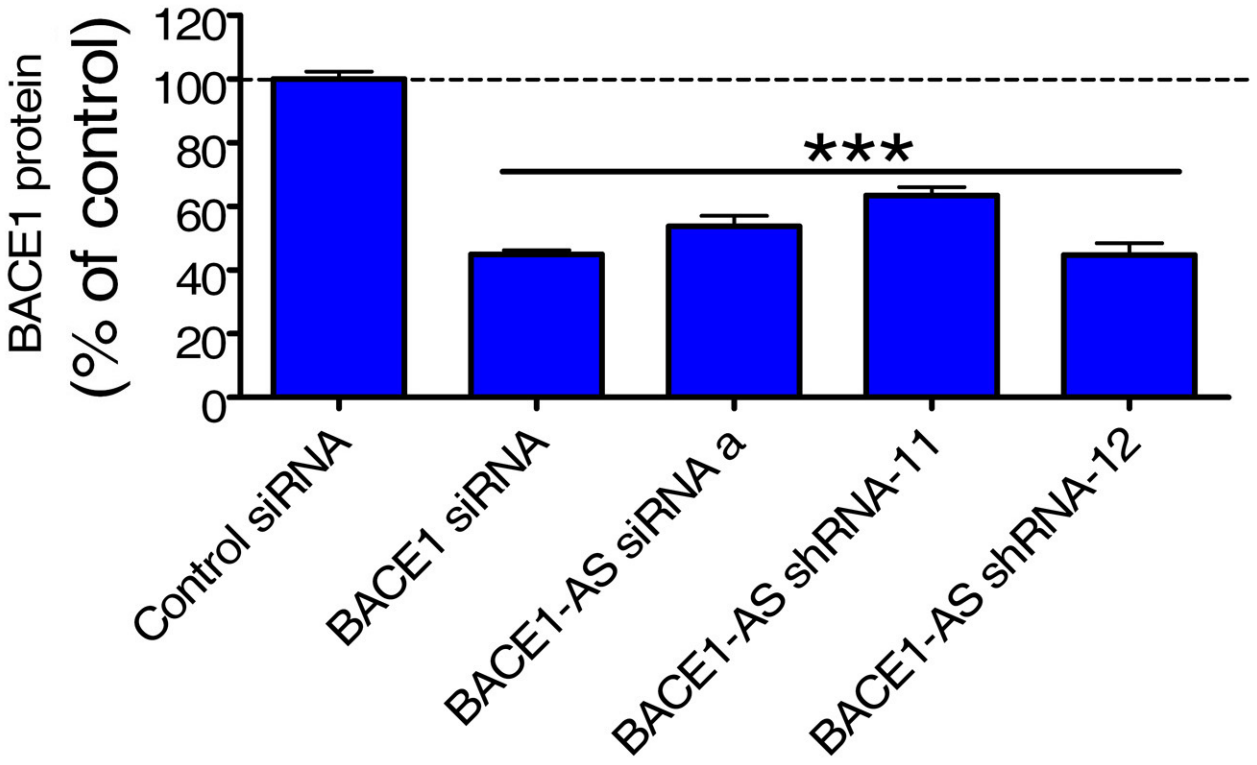


Figure S5. siRNA and shRNA mediated depletion of *BACE1-AS* causes BACE1 protein down-regulation:

HEK293T, C3 stable cell line with DiscoveRx vector, were used for siRNA and shRNA treatment against *BACE1*, *BACE1-AS* and control siRNA. After 72 h, BACE1 protein levels were measured using EFC assay. BACE1 protein was depleted in samples treated with siRNA or shRNA against *BACE1* or *BACE1-AS* confirming concordant regulation at the protein level.

Supplementary Figure-S6

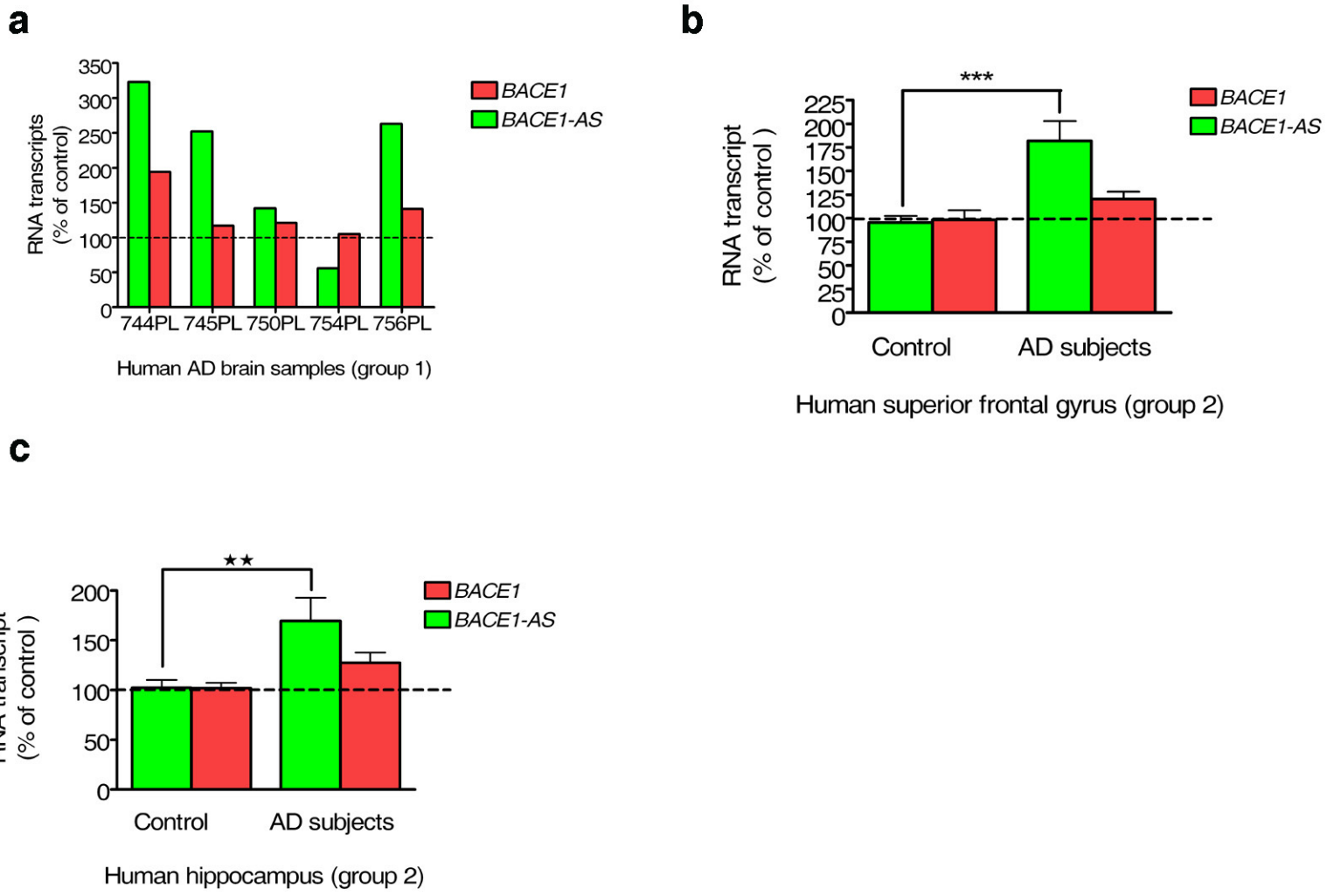


Figure S6. ***BACE1-AS*** **mRNA is elevated in AD individuals:**

(a) RNA samples, originated from parietal lobe of brain of 5 subjects with AD and 5 normal adult individuals were subjected to RT-PCR measurement. Relative quantity of *BACE1* and *BACE1-AS* in the brain of AD subjects calibrated to normal individuals and presented here as percentile to control. *BACE1-AS* expression was increased relatively by 2–3 times in AD brain samples.

(b) *BACE1-AS* transcript expression in superior frontal gyrus of 17 control individuals and 16 AD subjects. Up-regulation ($P < 0.0001$) of *BACE1-AS* was observed in AD individuals.

(c) *BACE1-AS* transcript expression in hippocampus of 11 control individuals and 13 AD subjects. Up-regulation ($P < 0.001$) of *BACE1-AS* was observed in AD subjects.

Human *BACE1-AS* sequence:

GGCTCACCGCAACCTCCACCGTCCTGAGTTAAAGTGATTCTCCTGTCTCAGCCCCCTGAGTA
GCTAGGATTACAGGCGTGCGCCACCACACCCAGCTAATTTTTGTACTTTTAGTAGAGATGGG
ATTTCACCCTGTTGGTCAGGCTGGTCTTGAACTCCTGACCTAGTGATCTGCCCACCTTGGCC
TCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCACGCCTGGCTAGGGGAAGAGTGCTTTA
AGAGCTCTGAGTAGAAGGGTCTAAGTGCAGACATCTTGGCTGTTGCTGAAGAATGTGACTCT
CACCGCCTCCCTCTGACACTGTACCATCTCTTTTACCCCCATCCTTAGTCCACTCACGGAGGAG
GCTGCCTTGATGGATTTGACTACAGCTTCAAACACTTTCTTGGGCAAACGAAGGTTGGTGGTG
CCACTGTCCACAATGCTCTTGTCATAGTTGTACTAAGAGGGAAAAGAGAGAGTTAAAAGAGT
CAAAAGGTTTTTGATGCTGGGCTCTGGGCAGTAGGGGGTTACTGCTGGGGCCCCAGCTGGG
TTGGCATCTTGGCTTTGGCACCTCCTAAGTGTACCTGCTTGGACAAGTTAACCTCTGTGCCT
CAGTTCCTTCATCTCTAAAGTGAGGATAAAAATAGCACCTACCTCAAAGGGTTATTGTAAGGA
TTAAATAAATCAGCAATGTAAAGCACTTAGAATCGTGCCCAGCAGAGAGAAGGCACTTGGTA
AATGTTTATTCTTGTTAATCTTGGGTGGGCAGGTAGTCTCCAAACTTGAAAAAAAAAAAAA

The sequence presented here is based on both 5', 3' RACE sequencing.
Red letters are potential overlap region with *BACE1* mRNA (104 bp).

BACE1-AS also aligns with several EST sequences from various cDNA libraries, including but not limited to CB960709, CF780740 and CB960760 from human placenta NIH-MGC collection, BP398116 from pancreatic islet collection[1], BF510444 and BF941666 from NCI-CGAP collection.

Reference:

1. Jin, L. et al. Expression profile of mRNAs from human pancreatic islet tumors. *J Mol Endocrinol* **31**, 519-28 (2003).

Mouse *BACE1*-AS sequences:

GTCTACGGATCAGGAGGAGAAAGTGAGATACTGGCCTCATAAAGCTGTGAGTGGGCATGGTGGCTCACACTGCCTGT
GGTCCCAGAACTTAGAAGGCTAAGGCATGAAGTTGTCAAGTTCAATGCTAACCTGGGCTACGTTCATGACCCCATCT
CAAAAAAAAAAAAAAAAAAAAAAAAGGCATCTCAATGCCAAGTCAACAGAGCAGTGGTTTGTAAGCGCCTGATGGGA
AGGATCTACGTATCTTTAGGGCTTGCTGAGGAGTGTGACTGTCACTGACCGAGGAGGCTGCCTTGATGGACTTGACG
GCAGCTTCAAATACTTTCTTGGGCAAGCGAAGGTTGGTGGTCCCACTGTCCACAATGCTCTTGTCGTAGTTGTACTG
AGGAGAGAGGGGGGGAGAGGTGGGAGGGGTGAATCTGACAGCTAAGCATCCTACTGGGGTTGGCATCTTAGCTTTCC
CATTTTGTATATGTGCGTCCTTGGGCACGTTGCTTAACCTCTGTGTACCTGTTCTCTTGTTCTAAATTGAAGATAAT
AACAGTGCCTACCTCAAAGGTTACCATAAGGATTACATAGGTCAAAAGGCTGAAAGCACTAAGTAGGTCCTGGCAGA
TCCCAGGCACTTGGTAAGTACTAACTTCGTCACCTTGTGCGGGTAGGTGTTCTCTAAATCTAAAGCAGAAATGCCTT
GTGTGGTATTTGTAACTTCTAGTACTTTTATGTAGTTATTCTCATTCACCATACATTATAATGGAGAGAAGGGTGGC
TCAGCACGCACAAAGCCATGAGCTTGAGCTCTGATGCTGCATGGTGGTATATGCCTGTAGAGAGACACTTGAGAGTC
AGAGAAAGGAGGACCAGGAGTTCAAGGTCATCTTTAGCTACCTAGCAAGTCTGAGGTCAGCGTGGGCTACATAAGAT
CCTCAAAAAAGCCAAAAAAGGGGGCTGTTGAGATAGGTAAGGGCACCTGCTACCAAGTCTGATGACCTGAGTTCAAC
CCCAGAGAGACCTACTAGTTACTGAAAATTGTCCTCTAACCTCCATACACAAGCTGTGGCACATGCCCCATCCTCAT
CCCAATAAATGTAAAACAAAATCTAGGGAAAGAGACCCTAAGTGTTGGCATTTGGGTATGCCAAGCATAACGACTAA
CACTTCATACATTGGCTTTGACCTTTACAGTCTGTGAGAACGCTTGTGTATTTCTACCTCTGCCTTGTAGATGAGGA
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TCCACCCTCATGGCTCTCACACACTGTGAGACTCCCCTTATGCTCACGAGAATCCCCTCCATCCCATTACCTCCTTG
CAGTCCATCTTGAGATCTTGACCATTGATTTCCACACGTACAATGATCACTTCATAATACCACTCCCGCCGGAGGGG
TGTGTACCAGAGACTGCCCGTGTATAGCGAGTGGTCGATACCACCAATGATCTAAAAGAAAAAGAGACAGACACCTA
TGTCCTAGCACAGAAGGAGAGCAAGTTACCCAAGACTAAATAATAAGATCAGCCATTTCTTGGGGTGCCAAGATTCT
CTCTAATCTCCCATCATGCCCCATGCATGGTAATATTTAGTTTCCTAAATGTGTTCAGGAGAAGAAACACATCGGGA
TTATTTGTATCAAAATCTATAGCCCTTGACCGAAAGTTATTTAAGCCCAAGCTAGTACAATAAACGTGGAATGAACT
GATTGTATGCTGCTTTCATACATTTTCTTGTCTTTGCTTGTTGGAGAATACTTGTTACTGTCCTAATTAATAAATGG
GCTTGAACTGAAAAAAAAAAAAA

The sequences shown here are based on the 3’ RACE sequencing.

Red letters are potential overlap region with *BACE1* mRNA.

Table-1: List of siRNA, primers and probes utilized for experiments and their sequences.
(F: Forward, R: Reverse, P: Probe)

#	Primer name	Application	Sequence
1	Mouse BACE1-AS_a -F	Real-time PCR	GTAGGCAGGGAAGCTAGTACTGA
2	Mouse BACE1-AS_a -R	Real-time PCR	AGAGGCTTGCAGTCCAGTTC
3	Mouse BACE1-AS_a -P	Real-time PCR	CCTGGAAGGAGAAACAG
4	Mouse BACE1-AS_b -F	Real-time PCR	TCTGCCTTGTAGATGAGGAGTCT
5	Mouse BACE1-AS_b -R	Real-time PCR	CCTGACTAGAGTCCTGCTGTTACTA
6	Mouse BACE1-AS_b -P	Real-time PCR	CTCAGGCTCCTCACTACAG
7	Mouse BACE1 target site	Real-time PCR	CCACAGACGCTCAACATCCTGGTGG
8	Mouse BACE1-AS siRNA	siRNA	GCTCGAGCTGCTATCAGTTTCCAAT
9	Human BACE1-AS siRNA_a	siRNA	CCCTCTGACACTGTACCATCTCTTT
10	Human BACE1-AS siRNA_b	siRNA	AGAAGGGTCTAAGTGCAGACATCTG
11	Human BACE1-AS siRNA_c	siRNA	CCAGAAGAGAAAGGGCACT
12	Human BACE1 siRNA_a	siRNA	GAGCCTTTCTTTGACTCTCTGGTAA
13	Human BACE1 siRNA_b	siRNA	CCACGGAGAAGTTCCCTGATGGTTT
14	BACEASCLON-F	3’RACE	TAGCGAGGTGACAGCGTAGA
15	BACEASCLON-R	5’RACE	GGGGAAGAAACTTAACCTTGG
16	HBAS-F	3’RACE	TTGGCTGTTGCTGAAGAATG
17	HBAS-R	5’RACE	CAGAGCCCACCATCAAAAAC
18	BACE1AS-F	3’RACE	TACCATCTCTTTTACCCCCATCCT
19	BACE1AS-R	5’RACE	AAGCTGCAGTCAAATCCATCAA
20	MBAS1-F	3’RACE	GCAGAGTGGCAACATGAAGA
21	MBAS1-R	5’RACE	TTTCTCCTCCTGATCCGTAGAC
22	MBASTest-F	3’RACE	GTCTACGGATCAGGAGGAGAAA
23	MBAS2-F	3’RACE	CCCTAAGTGTTGGCATTTGG
24	MBAS2-R	5’RACE	GGGCATGATGGGAGAATAGA
25	MBASTest-R	5’RACE	CCAAATGCCAACACTTAGGG
26	MBAS	siRNA	CAGAGAGACCTACTAGTTATT
27	MBAS	siRNA	TAACTAGTAGGTCTCTCTGTT
28	MBACE	siRNA	CACTGTGCGTGCCAACATTTT
29	MBACE	siRNA	AATGTTGGCACGCACAGTGTT
30	Negative control	siRNA	CCTCTCCACGCGCAGTACATT
31	Negative control	siRNA	TGTACTGCGCGTGGAGAGGTT
32	BACE1 shRNA-7	shRNA	CATTGGAGGTATCGACCACTCGCTGTACA
33	BACE1 shRNA-8	shRNA	TGAGGTTACCCCAGTCCTTCCGCATCA
34	BACE1 shRNA-9	shRNA	GGCTACAACATTCCACAGACAGATGAGTC
35	BACE1 shRNA-10	shRNA	GCTTTGTGGAGATGGTGGACAACCTGAGG
36	BACE1-AS shRNA-11	shRNA	TCCCTCTGACACTGTACCATCTCTTTTAC
37	BACE1-AS shRNA-12	shRNA	TAGAAGGGTCTAAGTGCAGACATCTTGGC
38	BACE1-AS shRNA-13	shRNA	TGCCCCCGCCAGAAGAGAAAGGGCACTTTG
39	BACE1-AS shRNA-14	shRNA	TGTTGCTGAAGAATGTGACTCTCACCGCC
40	BACE1-AS cloning-F	Cloning	AGACTCGAGTACAGGCGTGCGCCACCACA
41	BACE1-AS cloning-R	Cloning	ACTTGGATCCTTCCATTGCTGATTTATTTAATCC

42	BACE1 cloning-F	Cloning	TAGAATTCACCATGGCCCAAGCCCTGCCCTGGCT
43	BACE1 cloning-R	Cloning	ACTTGGATCCCTTCAGCAGGGAGATGTCATCAGCA
44	Human BACE1 target site	Real-time PCR	GCAGACGCTCAACATCCTGGTGGAT
45	Human APP target site	Real-time PCR	TTCATCATGGTGTGGTGGAGGTTGA
46	Mouse APP target site	Real-time PCR	GTGCCCCCAAGGCCTCATCATGTGT
47	Mouse BACE2 target site	Real-time PCR	TGGCCAAGCCATCAAGCTCTCTGGA
48	Human BACE2 target site	Real-time PCR	CTTGCCAAGCCATCAAGTTCTCTGG