

1 **Supplemental information**

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3 **Therapeutic potential of Bifidobacterium breve strain A1 for preventing**
4 **cognitive impairment in Alzheimer's disease**

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8 **Supplemental Material & Method**

9 **Microbiota analysis.**

10 DNA was extracted from cecal samples using the bead-beating method²⁰ and 16S
11 rRNA gene sequencing was performed as described previously¹. Briefly, The V3–V4
12 region of bacterial 16S rRNA genes were amplified via PCR with a TaKaRa Ex Taq
13 HS kit (TaKaRa Bio, shiga, Japan) and a primer set of Tru357F
14 (5'-CGCTCTTCCGATCTCTG TACGGRAGGCAGCAG-3') and Tru806R
15 (5'-CGCTCTTCCGATCTGAC GGACTACHVGGGTWTCTAAT-3'). The DNA
16 was amplified according to following program: 94 °C for 30sec; 30 cycles of 94 °C
17 for 30 sec, 50 °C for 30 sec and 72 °C for 5 min. PCR products was amplified with a
18 2nd primer set, which is adapted for the Illumina MiSeq (Illumina), by following
19 protocol: 94 °C for 30sec; 8 cycles of 94 °C for 30 sec, 50 °C for 30 sec and 72 °C for
20 5 min. Thereafter, equal amounts of amplicons from different samples were pooled

1 and removed primer-dimer by gel-extraction with the QIAquick PCR Purification Kit.
2 The pooled libraries were sequenced by Illumina MiSeq with a MiSeq v3 Reagent kit
3 (Illumina) in Morinaga Milk Company Co. Ltd.

4 The obtained sequences were filtered by the bowtie -2 program (ver 2-2.2.4) to
5 remove the reads mapped on PhiX174 sequence. Thereafter, the 3' region of each
6 read with a PHRED quality score of less than 17 was trimmed. Trimmed reads less
7 than 150 bp in length with an average quality score of less than 25 or those lacking
8 paired reads were also removed. The trimmed paired-end reads were combined by
9 the fastq-join script in EA-Utills (ver. 1.1.2–537). Potential chimeric sequences were
10 removed by reference-based chimera checking in USEARCH (ver. 5.2.32) and the
11 gold database (<http://drive5.com/otupipe/gold.tz>). The non-chimeric sequences were
12 analyzed in the QIIME software package version 1.8.0. The sequences were assigned
13 to operational taxonomic units (OTUs) by open-reference OTU picking with a 97%
14 pairwise identity threshold and the Greengenes reference database
15 (ftp://greengenes.microbio.me/greengenes_release/gg_13_5/gg_13_8_otus.tar.gz).

16

17 **Short-chain fatty acid (SCFA) analysis.**

18 The plasma samples were prepared for gas chromatography-mass spectroscopy
19 (GC-MS) analysis as described previously² with modifications. 90 µL of plasma was
20 mixed with 2 µL of 2.9 mM [2H3] acetate (Cambridge Isotope Laboratories,

1 Tewksbury, MA, U.S.A.) as an internal standard, deproteinized with 2 μ L of 1
2 mg/ μ L 5-sulfosalicylic acid and centrifuged at $2000 \times g$ for 10 min. The supernatant
3 was then collected and mixed with 2 μ L of 35% HCl followed by 600 μ L of diethyl
4 ether. The solution was vortexed for 30 min and then centrifuged for 10 min at 1200
5 $\times g$ at room temperature. The ether layer was collected and 1.6 μ L of
6 1-(tert-butyldimethylsilyl) imidazole (Tokyo Chemical Industry, Tokyo, Japan) was
7 added. The solution was heated at 60 $^{\circ}$ C for 30 min, and then cooled and transferred
8 to a glass vial for GC-MS analyses. Analysis was conducted with the
9 GC-MS-QP2010 Ultra (Shimadzu, Kyoto, Japan) using electron ionization mode and
10 a CP-Sil 8CB-MS column (30 m \times 0.25 mm internal diameter \times 0.25 μ m; J&W
11 Scientific, Folsom, CA, U.S.A.) for GC separation under the following thermal
12 conditions: 40 $^{\circ}$ C for 1 min, 70 $^{\circ}$ C/min until 60 $^{\circ}$ C, 10 $^{\circ}$ C/min until 110 $^{\circ}$ C, and
13 70 $^{\circ}$ C/min until 250 $^{\circ}$ C for 1 min. The mass spectrometer was set in selected ion
14 monitoring mode at m/z 117 for acetate, m/z 120 for [2H3] acetate, m/z 131 for
15 propionate, and m/z 125 for butylate.

16

17 **Quantitative RT-PCR**

18 Real-time quantitative RT-PCR was performed using PrimeScript RT reagent Kit
19 (Takara Bio, shiga, Japan) and SYBR Premix Ex Taq II (Takara Bio). The level of
20 target gene expression was normalized against expression of a housekeeping gene,

1 glycerinaldehyde-3-phosphate dehydrogenase (GADPH), in each sample. PCR primers
2 were shown in supplemental Table 3.

3

4 **A β 1-42 analysis**

5 Photo-Induced Cross-Linking of Unmodified Proteins (PICUP) and SDS-PAGE
6 were performed as described previously³. A β separated on SDS-PAGE gels were
7 silver-stained using Silver Quest Silver Staining kit (Invitrogen) according to
8 manufacture instruction.

9

10 **Reference for Supplemental Information**

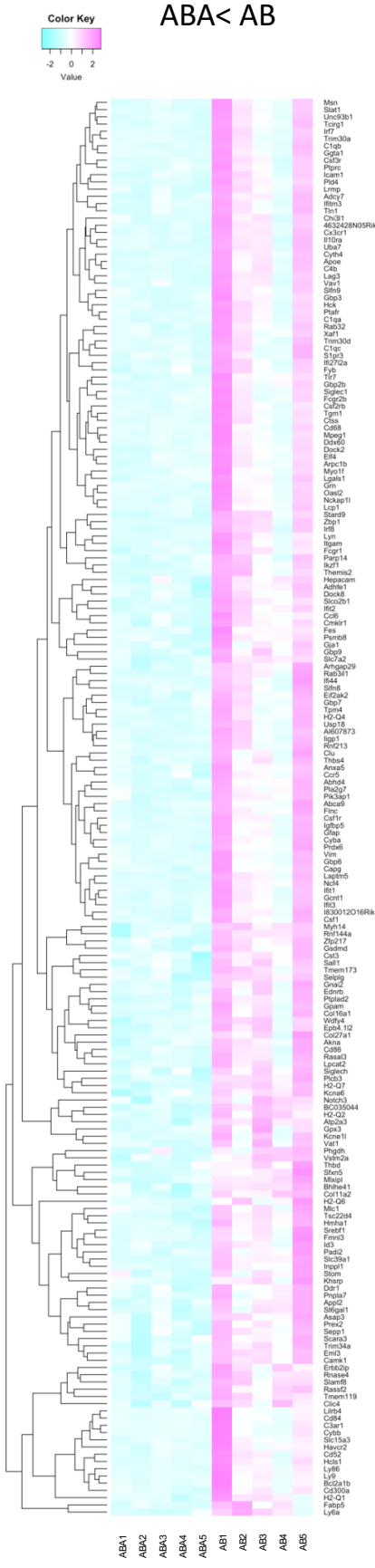
- 11 1. Odamaki, T. *et al.* Age-related changes in gut microbiota
12 composition from newborn to centenarian: a
13 cross-sectional study. *BMC Microbiol.* **16**, 90 (2016).
- 14 2. Tsukahara, T. *et al.* High-sensitivity detection of
15 short-chain fatty acids in porcine ileal, cecal, portal and
16 abdominal blood by gas chromatography-mass
17 spectrometry. *Anim. Sci. J.* **85**, 494–498 (2014).
- 18 3. Rahimi, F., Maiti, P. & Bitan, G. Photo-Induced
19 Cross-Linking of Unmodified Proteins (PICUP) Applied
20 to Amyloidogenic Peptides. *J. Vis. Exp.* (2009).

1 doi:10.3791/1071

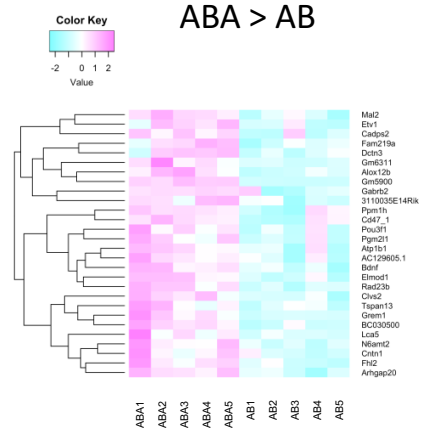
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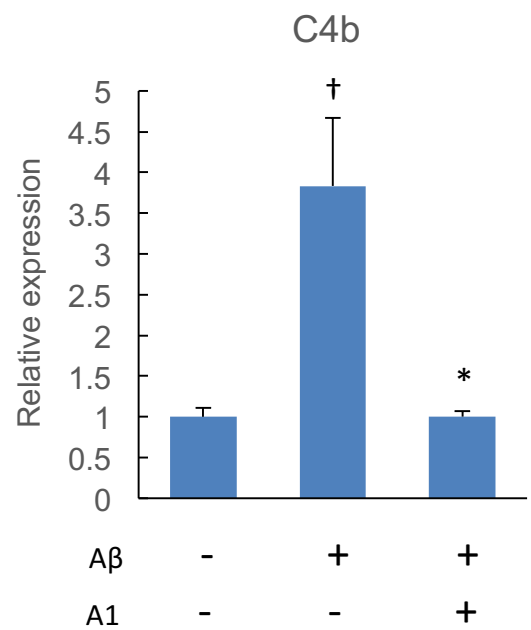
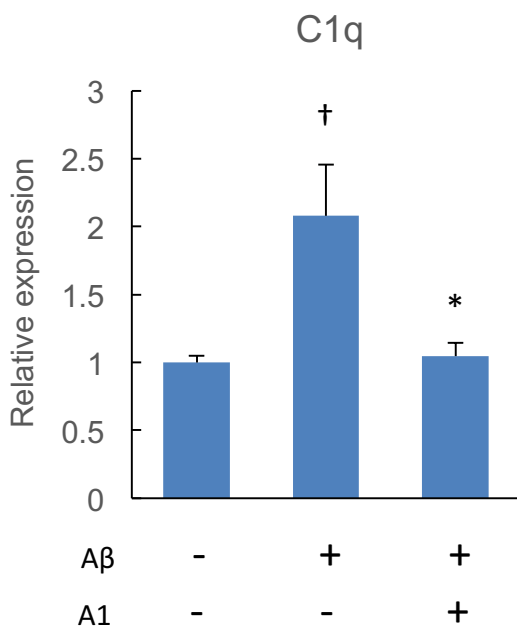
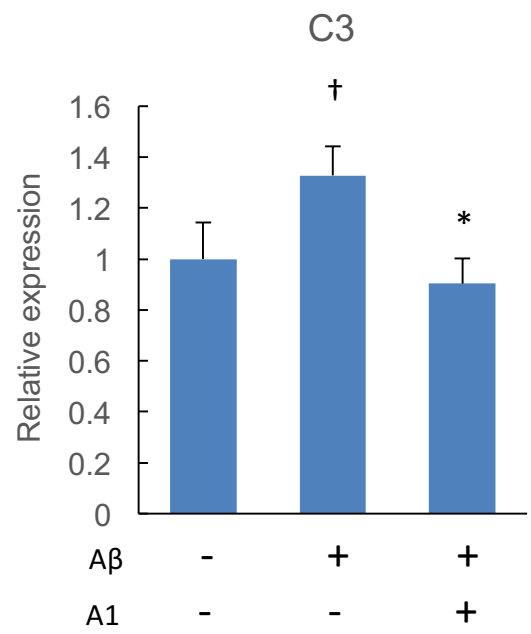
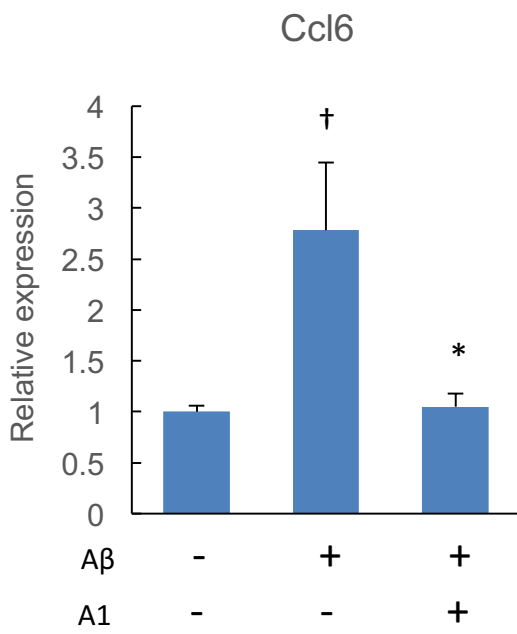
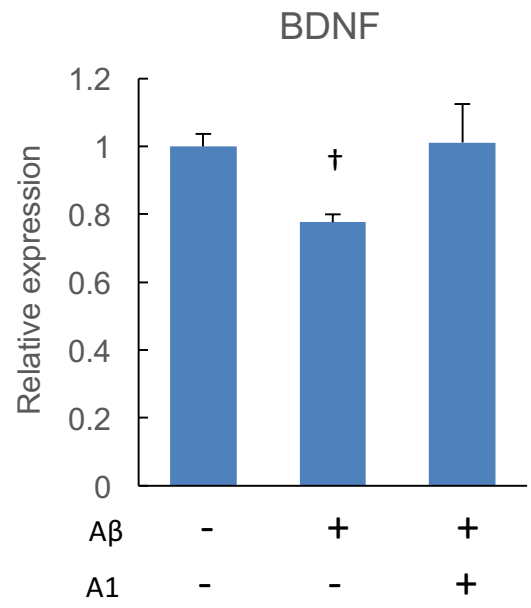
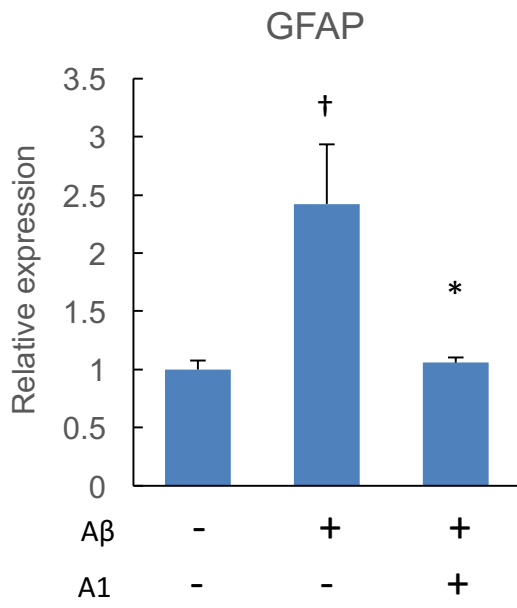
d



1 **Supplemental Figure 1.**

2 (a–b) Heatmaps of the significantly modulated gene expression ($p < 0.05$ and
3 $FDR < 0.05$ up- or downregulation) in hippocampus of $A\beta$ injected mice (lanes AB1–
4 AB5) in comparison with sham-operated mice (lanes SH1–SH5). We detected
5 changes in the expression of 305 genes (247 up-regulated, 58 down-regulated). (c–d)
6 Heatmaps of the significantly modulated gene expression in hippocampus of $A\beta$
7 injected mice administrated *B. breve* A1 (lanes ABA1–ABA5) compared with
8 non-administrated mice (lanes AB1–AB5).
9

Supplemental Fig 2



1 **Supplemental Figure 2. Verification of RNA-seq data by quantitative RT-PCR**

2 **analysis.**

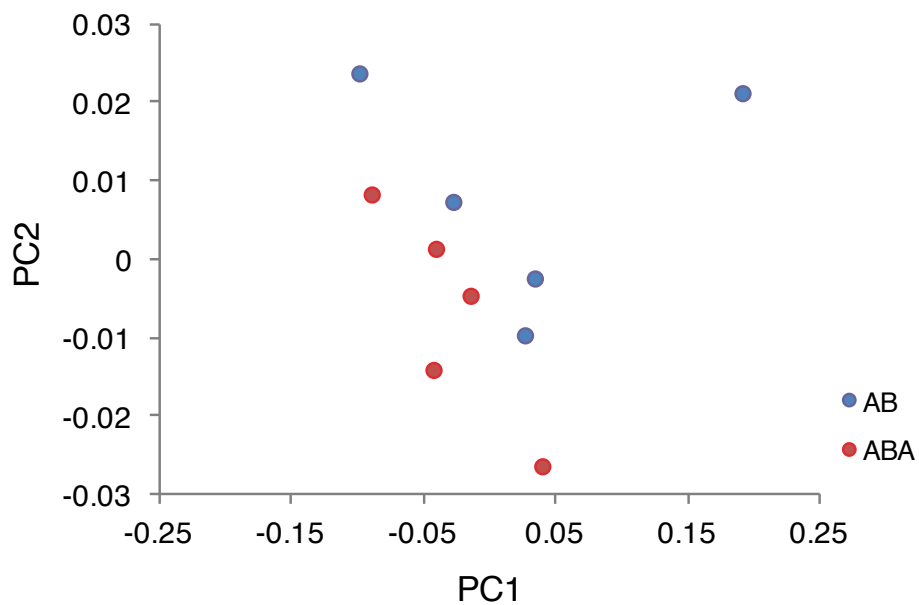
3 mRNA expression of Gfap (A), Ccl6 (B), C1qa (C), C3 (D), C4b (E) and Bdnf (F) in

4 hippocampus. n=5 mice in each group. †P<0.05 vs. SH, *P<0.05 vs. AB.

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6

Supplemental Fig 3



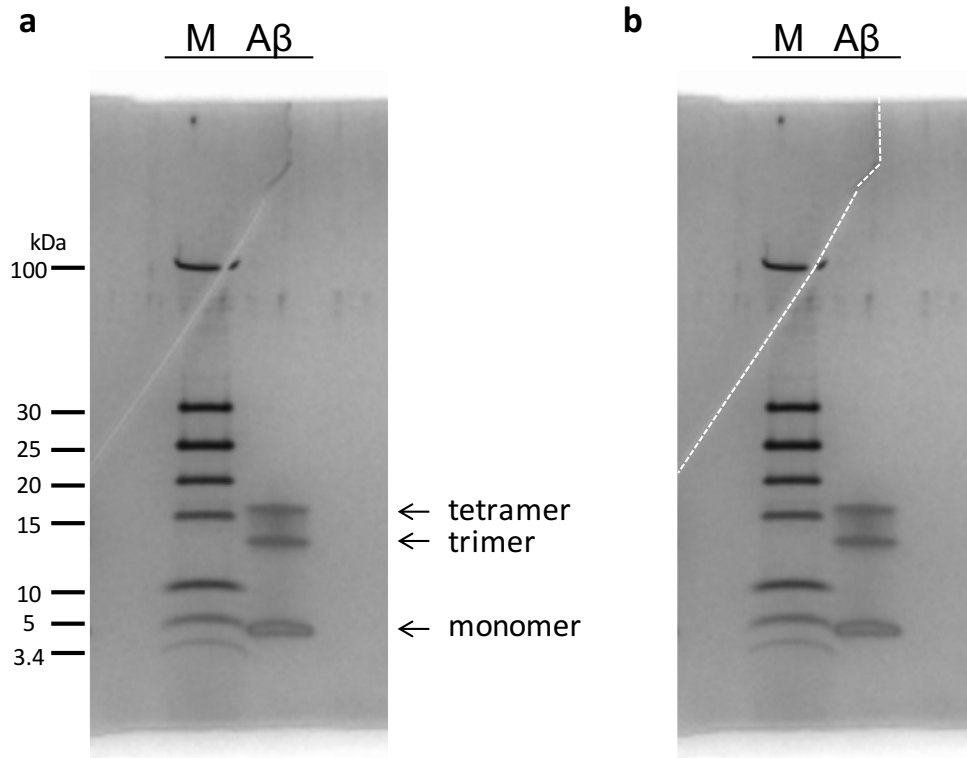
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2 **Supplemental Figure 3.**

3 Bray-Curtis dissimilarity based principal coordinates analysis (PCoA) was
4 performed on 16S rRNA genes at phylum level. There was no significant difference
5 between A β injected mice (AB) and A β injected mice administrated *Bifidobacterium*
6 *breve* strain A1 (ABA) by using two-way permutation Manova.

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3 **Supplemental Figure 4.**

4 (a) SDS-PAGE and silver-staining are performed to visualize cross-linked A β peptide.

5 M: protein ladder marker, A β : A β 1-42 solution. A β 1-42 we used in this study

6 produced three bands consistent with monomers, trimers and tetramers. (b) The

7 cropped location in the SDS-PAGE gel was indicated by white dotted line.

8

Supplemental Table 1: List of gene expression profile in hippocampus of AD model mice by *Bifidobacterium breve* A1 treatment using RNA-seq analysis.

	group	number of genes	gene name
SH vs AB	SH>AB	58	Cntn1, Lrrtm4, Grin2b, Bdnf, Ehd3, Trpc4, Ap2b1, Lrrn1, Ta9_1, D3Bwg0562e, Ate1, Cisd1, Uqcrc2, Ppa1, Usp12, Elmod1, Civs2, Gcnt2, Dapk1, Pip5k1b, Atp2b3, Cops5, Atp5a1, Wdr61, Gpr22, Ldlr, Dlg3, Hspa4, Ipk2c, Cycs, Emc2, Fdtt1, Crbn, AB30018L16Rik_1, Gm21955, Paqr9, Dctn3, Nrip3, Atp1b1, Slc16a7, Ap3b1, Fdps, Gareem, Cers6, Hmgcr, Rad23b, Grasp, D5Erd579e, Atp5b, Fam65b, Dnm11, Fam84a, AC129605.1, Atp6ap2, Mdh1, Ddn, Pls3, Wast1
	SH<AB	247	Hmha1, Col11a2, Selplg, Slco2b1, Rappgef3, Ccr5, Sspo, Oasl2, Pld4, Glul, Epb4.1i2, Ifi3, Col16a1, Laptm5, Tsc22d4, Sall1, Sepp1, Lag3, C4b, Col24a1, Fes, Gmp1, Rnf213, Fcgr1, Csf3r, Trim34a, BC030499, Cx3cr1, Tlr9, Cyth4, Rassf3, Ii17ra, Itgam, Cit, Inpp5d, Pik3ap1, Rps6ka1, Trim30a, Unc93b1, Matn4, Scara3, Jak3, Stard9, Parp14, Csf1r, Kcna6, Tlr7, Aifm3, H2-Q7, Slnf8, Irf7, Myo1f, 4632428N05Rik, Slc39a1, Nrpb2, Icam1, Tnfrsf11a, Gpx3, Fyb, Rora, Fgd2, Plcb3, Tcrg1, Slc11a1, Zbp1, Lpcat2, Itpril2, Dock2, Iglp, Oas1a, Rnf144a, Prdx6, H2-Q2, Sparc, Rasgef1b, Msn, Hepacam, I830012O16Rik, Ifi1, Trim30d, Irf8, Rasal3, Cmkir1, Capg, Nckap11, Ggta1, Tifab, Gatm, Bhlhe41, Pnpla7, Gbp2b, Vwa1, Lcp1, Gigyf1, Atp1a2, AF251705, Mef2c, Gm15631, Zfp361f, Igsf9b, Usp18, Siglec1, Adcy7, Lyn, Uba7, Ifitm3, Anxa3, Ptpcr, Ly86, Amy1, Grn, Stom, Plekha4, Prex2, Cadm4, Tnfrsf23, Rnase1, C1qc, Appl2, Xaf1, Prosl, Gja1, Slnf9, Vav1, Cst3, Herc6, Gsdmd, Fhod3, Camk1, Ii10ra, Sipa1, C1qa, Myo1c, Ifi2, Aldh1l1, Pygm, Rasgef1c, Mpeg1, C1qb, Tgm1, Apoe, Ptpn6, Cyba, Cd84, Itgb2, Mafk, Sparcl1, Finc, Rims3, Ddx60, Cst2ra, Trim56, Sfxn5, Gnai2, Notch3, Ptafr, Gbp3, Ehd2, Zcchc24, Ifi44, Ccl19, Irf5, Tmem173, Cd14, A1607873, Necap2, Thbs4, H2-Q4, Renbp, Hk3, Asap3, Fcgr3, Zfp361f, Ly9, Gfap, Sreb1, Rnase4, Hcls1, Wdfy4, Lair1, Tnfrsf1b, Dnm2, Chl31, Nfami, Snhg11, Grpp1, Inpp1f, Rarg, Plcd3, Ctss, Gjb6, Ncf4, Rin2, Slc15a3, Plekho1, Arhgap4, Garm3, Abca9, Cd53, Farp1, 2610507O1Rik, Ifi2712a, Gm2026, Cited4, Sfi1, H2-Q6, Coro6, Ttyh1, Btk, Flnb, Liril4, H2-T10, Cbin2, Cd33, Sgpl1, Clec4a3, Ankrd34c, Mical1, Clu, Slc44a2, Atp1b2, Itpr2, Cyp2j9, Iglbp5, Vim, Cux1, Arhgap30, Slamf8, Sbnoc2, Gnat13, Irf9, Osmr, Psmb8, Ms4a6c, C3ar1, Oplah, Cux2_1, Traf3ip3, Ch25h, Hck, Vasp, Tgfb2, Ii33, R3hdm2, Skap2, Cybb, Itgal
SH vs ABA	SH>ABA	1	Gm6472
	SH<ABA	1	Pole4
AB vs ABA	AB>ABA	196	Laptm5, Slco2b1, Plk4, Cyth4, C4b, Gbp2b, Tsc22d4, Hmha1, H2-Q4, H2-Q7, Ifi3, Oasl2, H2-Q2, Plcb3, Finc, Lag3, Col11a2, Ly6a, I830012O16Rik, Cx3cr1, Selplg, Rnf213, Csf1r, Ifi2, Slc7a2, Rassf2, Ly86, Ncf4, Ifi1, Col16a1, Themis2, Tmem119, Epb4.1i2, Nckap11, Zbp1, Slc39a1, Itgam, Cyba, Pla2g7, Pik3ap1, Camk1, Tlr7, 4632428N05Rik, Rab3il1, Gbp9, Psmb8, Uba7, Gsdmd, Wdfy4, Lcp1, Thbd, Tcrg1, Rasal3, Unc93b1, Ikbz1, Gbp3, Myo1f, Fcgr1, Csf3r, Ptpad2, Pad12, Usp18, Irf8, Trim34a, Gfap, Erbb2ip, H2-Q6, Fabp5, Ddx60, Irf7, Xaf1, Stat1, Slc15a3, Cybb, Adhfe1, Dock2, Abhd4, BC035044, St6gal1, Akna, Sreb1f, Arpc1b, A1607873, Atp2a3, Eif2ak2, Lpcat2, Msn, Anxa5, C1qa, Capg, Gpam, Zfp217, Tpm4, Ptpcr, Gpx3, Tmem173, Dock8, Stard9, Siglec1, Apoe, Cd84, Ednrb, Mxip1, Tgm1, Iigp1, Ifi44, Gcnt1, Arhgap29, Gbp6, Csf2rb, Slnf8, Parp14, Sall1, Ccl6, Notch3, Cst3, Fes, Fcgr2b, Vatl1, Cd300a, Rnf144a, Ii10ra, Lyn, Rnase4, Abca9, Hck, Csf1, Icam1, Lrmp, Grn, Fyb, Sfxn5, Gnai2, Ggta1, Ddr1, Asap3, Hepacam, Ptafr, Cd68, Hcls1, Inpp1f, Ifitm3, Adcy7, Kcne1f, Thbs4, Vav1, Vim, C3ar1, Mlcl, Gbp7, Trim30d, Slamf8, Phgdh, C1qb, Trim30a, Liril4, S1pr3, Myh14, Scara3, Vstm2a, Eml3, Ccr5, Col27a1, Havcr2, Sepp1, Id3, Prex2, Fmnl3, Ctss, Cmkir1, Appl2, Stom, Elf4, Cd86, Iglbp5, Rab32, Clu, Cd52, Prdx6, Clic4, Mpeg1, Gja1, C1qc, Bcl2a1b, Bhlhe41, Tin1, Pnpla7, H2-Q1, Slnf9, Kherp, Kcna6, Chl31, Ly9, Ifi2712a, Lgals1, Siglech
	AB<ABA	28	Gm5900, Gm6311, Elmod1, Grem1, Arhgap20, Bdnf, 3110035E14Rik, Civs2, Alox12b, Mal2, N6amt2, Pgm21, Atp1b1, Rad23b, Cd47_1, Cntn1, Lca5, Dctn3, AC129605.1, Fhl2, Gabrb2, Pou3f1, BC030500, Cadps2, Tspan13, Ppm1h, Etv1, Fam219a,
	(AB>ABA) ∩ (SH<AB)	130	Laptm5, Slco2b1, Plk4, Cyth4, C4b, Gbp2b, Tsc22d4, Hmha1, H2-Q4, H2-Q7, Ifi3, Oasl2, H2-Q2, Plcb3, Finc, Lag3, Col11a2, I830012O16Rik, Cx3cr1, Selplg, Rnf213, Csf1r, Ifi2, Ly86, Ncf4, Ifi1, Col16a1, Epb4.1i2, Nckap11, Zbp1, Slc39a1, Itgam, Cyba, Pik3ap1, Camk1, Tlr7, 4632428N05Rik, Psmb8, Uba7, Gsdmd, Wdfy4, Lcp1, Tcrg1, Rasal3, Unc93b1, Gbp3, Myo1f, Fcgr1, Csf3r, Usp18, Irf8, Trim34a, Gfap, H2-Q6, Ddx60, Irf7, Xaf1, Slc15a3, Cybb, Dock2, Sreb1f, A1607873, Lpcat2, Msn, C1qa, Capg, Ptpcr, Gpx3, Tmem173, Stard9, Siglec1, Apoe, Cd84, Tgm1, Ifi44, Slnf8, Parp14, Sall1, Notch3, Cst3, Fes, Rnf144a, Ii10ra, Lyn, Rnase4, Abca9, Hck, Icam1, Grn, Fyb, Sfxn5, Gnai2, Ggta1, Asap3, Hepacam, Ptafr, Hcls1, Inpp1f, Ifitm3, Adcy7, Thbs4, Vav1, Vim, C3ar1, Trim30d, Slamf8, C1qb, Trim30a, Liril4, Scara3, Ccr5, Sepp1, Prex2, Ctss, Cmkir1, Appl2, Stom, Iglbp5, Clu, Prdx6, Mpeg1, Gja1, C1qc, Bhlhe41, Pnpla7, Slnf9, Kcna6, Chl31, Ly9, Ifi2712a
	(AB<ABA) ∩ (SH>AB)	8	AC129605.1, Atp1b1, Bdnf, Civs2, Cntn1, Dctn3, Elmod1, Rad23b

The table presents the list of shared and unique DE genes in SH vs AB, SH vs ABA and/or AB vs ABA. SH: sham-operated mice; AB: Aβ25-35 injected mice; ABA: mice treated with Aβ and *Bifidobacterium breve* A1.

Supplemental Table 2: List of gene expression profile in hippocampus of sham-operated mice by *Bifidobacterium breve* A1 treatment using RNA-seq analysis.

	group	number of genes	gene name
SH vs SHA	SH>SHA	0	-
	SH<SHA	1	Cers1

The table presents the list of shared and unique DE genes in SH vs SHA. SH: sham-operated mice; SHA: sham-operated mice treated with *Bifidobacterium breve* A1.

Supplemental Table 3: PCR primers used in Supplemental Figure 2

	PCR primer
glyceraldehyde-3-phosphate dehydrogenase (Gapdh)	(forward) AAATGGTGAAGGTCGGTGTG
	(reverse) TGAAGGGGTCTGTTGATGG
glial fibrillary acidic protein (Gfap)	(forward) GACCAGCTTACGGCCAACAG
	(reverse) TCTATACGCAGCCAGGTTGTTCTC
chemokine (C-C motif) ligand 6 (Ccl6)	(forward) AGAAACTCCAAGACTGCCATTCA
	(reverse) TTTACATGGGATCTGTGTGGCATA
complement component 1, q subcomponent, alpha polypeptide (C1qa)	(forward) CATCCAGTTTGATCGGACCAC
	(reverse)AGACATCTTCAGCCACTGTCCATAC
complement component 3 (C3)	(forward) CCTGTAGTGCGCTGGCTCAA
	(reverse) CAAGTCCTTATGGTCAGGGACATC
complement component 4B (C4b)	(forward) ACCGTTACGTGAGTCACTTTGAGA
	(reverse) ATACAGGACAGCACTGGATGG
brain derived neurotrophic factor (Bdnf)	(forward) TCAAGTTGGAAGCCTGAATGAATG
	(reverse) CTGATGCTCAGGAACCCAGGA