

Selective Vulnerability of the Aging Cholinergic System to Amyloid Pathology Revealed by Induced APP Overexpression

Kan Xie¹, Devon Ryan^{1§}, Susanne Schröder¹, Lena Freund^{1&}, Stefan Bonn², Yu Zhou³ and Dan Ehninger^{1*}

*Correspondence should be addressed to: Dan.Ehninger@dzne.de

¹Translational Biogerontology Lab, German Center for Neurodegenerative Diseases (DZNE), Venusberg-Campus 1/99, 53127 Bonn, Germany

²Institute of Medical Systems Bioinformatics, Center for Biomedical AI (bAlome), Center for Molecular Neurobiology (ZMNH), Center for Translational Immunology (HCTI), German Center for Child and Youth Health (DZKJ), University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

³School of Life Sciences and Health, University of Health and Rehabilitation Sciences, Qingdao, Shandong 266021, China

[§]Present address: Genedata AG, Margarethenstr. 38, 4053 Basel, Switzerland

[&]Present address: Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstr. 6, 52074 Aachen, Germany

Supplementary Table 1. Comparison of APP-associated differentially expressed genes (DEGs) uncovered in the present study with a systematic meta-analysis of human AD transcriptomic datasets

Gene symbol	DEG in the present study	DEG in human AD by Yuen et al., 2020
<i>4632428N05Rik</i>	True	False
<i>Adam33</i>	True	True
<i>Anxa4</i>	True	False
<i>App</i>	True	False
<i>Arl11</i>	True	True
<i>Asb10</i>	True	True
<i>Aspg</i>	True	True
<i>Atp13a4</i>	True	False
<i>B430306N03Rik</i>	True	False
<i>C1qa</i>	True	True
<i>C1qb</i>	True	False
<i>C1qc</i>	True	True
<i>C3ar1</i>	True	True
<i>C4b</i>	True	True
<i>Cckbr</i>	True	True
<i>Ccl3</i>	True	False
<i>Ccl4</i>	True	True
<i>Ccl6</i>	True	False
<i>Cd14</i>	True	True
<i>Cd180</i>	True	True
<i>Cd37</i>	True	True
<i>Cd44</i>	True	True
<i>Cd52</i>	True	False
<i>Cd53</i>	True	True
<i>Cd68</i>	True	False
<i>Cd72</i>	True	False
<i>Cd84</i>	True	True
<i>Cd9</i>	True	False
<i>Clec7a</i>	True	True
<i>Creg1</i>	True	False
<i>Csf1r</i>	True	True
<i>Csf3r</i>	True	True
<i>Cst7</i>	True	True
<i>Ctsd</i>	True	False
<i>Ctsl</i>	True	True
<i>Ctss</i>	True	True
<i>Ctsz</i>	True	False
<i>Ctnbp2</i>	True	False
<i>Cx3cr1</i>	True	False
<i>Cxcl5</i>	True	False
<i>Dclre1b</i>	True	True
<i>Derl1</i>	True	True
<i>Dusp14</i>	True	True
<i>Fam46c</i>	True	False
<i>Fcer1g</i>	True	True

<i>Fcgr2b</i>	True	True
<i>Fcgr3</i>	True	False
<i>Fcrls</i>	True	False
<i>Fgr</i>	True	True
<i>Flnc</i>	True	True
<i>Gfap</i>	True	True
<i>Ggta1</i>	True	True
<i>Glpr2</i>	True	True
<i>Gpr183</i>	True	True
<i>Gpr65</i>	True	True
<i>Grn</i>	True	False
<i>Gusb</i>	True	False
<i>Hexa</i>	True	False
<i>Hexb</i>	True	True
<i>Irf5</i>	True	True
<i>Irf8</i>	True	True
<i>Itgax</i>	True	True
<i>Itgb2</i>	True	True
<i>Kirrel2</i>	True	True
<i>Klhl6</i>	True	True
<i>Krt20</i>	True	False
<i>Lag3</i>	True	False
<i>Lair1</i>	True	True
<i>Laptm5</i>	True	True
<i>Lcp1</i>	True	True
<i>Lgals3bp</i>	True	False
<i>Lilrb4</i>	True	True
<i>LOC101056001</i>	True	False
<i>Ly86</i>	True	True
<i>Mamdc2</i>	True	False
<i>Man2b1</i>	True	False
<i>Milr1</i>	True	False
<i>Mpeg1</i>	True	False
<i>Msn</i>	True	True
<i>Myo1f</i>	True	True
<i>NA</i>	True	False
<i>Ncf1</i>	True	False
<i>Niacr1</i>	True	False
<i>Nrsn1</i>	True	True
<i>Olfml3</i>	True	True
<i>Oprk1</i>	True	True
<i>Osmr</i>	True	True
<i>P2ry13</i>	True	False
<i>Pdcd1</i>	True	True
<i>Plek</i>	True	True
<i>Plxdc2</i>	True	True
<i>Pou3f3os</i>	True	False
<i>Prnp</i>	True	False
<i>Ptpn6</i>	True	True
<i>Ptprc</i>	True	True
<i>Ptprr</i>	True	True
<i>Pycard</i>	True	True
<i>R3hdm1</i>	True	True

<i>Rac2</i>	True	True
<i>Rdh11</i>	True	True
<i>Renbp</i>	True	True
<i>S100a16</i>	True	False
<i>Samsn1</i>	True	True
<i>Serpina3n</i>	True	False
<i>Slamf9</i>	True	False
<i>Slc11a1</i>	True	True
<i>Slc14a1</i>	True	True
<i>Slc15a2</i>	True	True
<i>Slc15a3</i>	True	True
<i>Slc5a7</i>	True	True
<i>St14</i>	True	True
<i>St6galnac2</i>	True	False
<i>Stac3</i>	True	False
<i>Stard4</i>	True	False
<i>Syngn2</i>	True	False
<i>Tbxas1</i>	True	True
<i>Tlr13</i>	True	False
<i>Tlr2</i>	True	True
<i>Tmem176a</i>	True	True
<i>Tnfaip8l2</i>	True	False
<i>Tnfrsf1a</i>	True	True
<i>Tnfsf8</i>	True	False
<i>Trem2</i>	True	True
<i>Trem12</i>	True	False
<i>Trpm1</i>	True	True
<i>Tyrobp</i>	True	True
<i>Wfdc17</i>	True	False

Supplementary Table 2. Gene expression levels of mouse brain cell lineage markers

Differential expression analysis was conducted to compare brain transcriptomic profiles between APP 6→18mo (2 males and 2 females), APP 12→24mo (2 males and 2 females), and age-matched controls (2 male and 1 female 18-month-old controls; 2 male and 2 female 24-month-old controls). A false discovery rate (FDR) threshold of 0.05 was applied. Statistically significant comparisons are highlighted in bold. NA = p-value not available.

Cell type	Specific cell type	Gene	P-value (Age)	P-value (APP)	P-value (AgexAPP)
Glia	Astrocytes	<i>Aldh1l1</i>	0.9998	0.9492	0.9999
		<i>Aldoa</i>	0.9998	0.8978	0.9999
		<i>Aldob</i>	0.9998	0.9525	0.9999
		<i>Aldoc</i>	0.9998	0.8041	0.9999
		<i>Aqp4</i>	0.9998	0.1118	0.9999
		<i>Coro1a</i>	0.9998	0.8978	0.9999
		<i>Gfap</i>	0.9998	<0.0001	0.9999
		<i>Igfbp3</i>	0.9998	0.9924	0.9999
		<i>S100b</i>	0.9998	0.5919	0.9999
	Microglia	<i>Aif1</i>	0.9998	0.6631	0.9999
		<i>C1qa</i>	0.9998	<0.0001	0.9999
		<i>C1qb</i>	0.9998	0.0002	0.9999
		<i>Cd14</i>	0.9998	<0.0001	0.9999
		<i>Cd40</i>	NA	0.7956	0.9999
		<i>Cd68</i>	0.9998	<0.0001	0.9999
		<i>Cst3</i>	0.9998	0.8654	0.9999
		<i>Cx3cr1</i>	0.9998	0.0123	0.9999
		<i>Emr1</i>	0.9998	0.2944	0.9999
		<i>Itgam</i>	0.9998	0.0901	0.9999
		<i>Nos2</i>	0.8248	0.9369	0.9999
		<i>P2ry12</i>	0.9998	0.3255	0.9999
		<i>Ptprc</i>	0.9998	0.0092	0.9999
		<i>Pycard</i>	0.9998	0.0433	0.9999
		<i>Spi1</i>	0.9998	0.0647	0.9999
		<i>Tlr2</i>	0.9998	0.0139	0.9999
		<i>Tmem119</i>	0.9998	0.9984	0.9999
	Oligodendrocytes	<i>Cldn11</i>	0.9998	0.5186	0.9999
		<i>Cnp</i>	0.9998	0.7725	0.9999
		<i>Cspg4</i>	0.9998	0.9866	0.9999
		<i>Galc</i>	0.9998	0.8412	0.9999
		<i>Mag</i>	0.9998	0.8212	0.9999
		<i>Mbp</i>	0.9998	0.8436	0.9999
		<i>Mog</i>	0.9998	0.8428	0.9999

		<i>Olig1</i>	0.9998	0.9966	0.9999
		<i>Olig2</i>	0.9998	0.9866	0.9999
		<i>Pdgfra</i>	0.9998	0.8731	0.9999
	Radial glia	<i>Fabp7</i>	0.9998	0.9905	0.9999
		<i>Tnc</i>	0.9998	0.9866	0.9999
		<i>Vim</i>	0.9998	0.2078	0.9999
Neurons	Neural progenitor cells	<i>Ascl1</i>	0.9998	0.9009	0.9999
		<i>Cntnap1</i>	0.9998	0.9966	0.9999
		<i>Cdh1</i>	0.9998	0.7956	0.9999
		<i>Cdh2</i>	0.9998	0.8654	0.9999
		<i>Fut4</i>	NA	0.9785	0.9999
		<i>Msi1</i>	0.9998	0.9782	0.9999
		<i>Msi2</i>	0.9998	0.9866	0.9999
		<i>Nes</i>	0.9998	0.9492	0.9999
		<i>Notch1</i>	0.9998	0.9534	0.9999
		<i>Otx2</i>	0.9998	0.9292	0.9999
		<i>Pax3</i>	NA	0.5508	0.9999
		<i>Pax6</i>	0.9998	0.9083	0.9999
		<i>Smarca4</i>	0.9998	0.8640	0.9999
		<i>Sox1</i>	0.9998	0.9994	0.9999
		<i>Sox2</i>	0.9998	0.8519	0.9999
		Immature neurons	<i>Dcx</i>	0.9998	0.9429
	<i>Eno2</i>		0.9998	0.7846	0.9999
	<i>Ncam1</i>		0.9998	0.8654	0.9999
	<i>Neurod1</i>		0.9998	0.7824	0.9999
	<i>Stmn1</i>		0.9998	0.9045	0.9999
	<i>Tbr1</i>		0.9998	0.9740	0.9999
	Mature neurons	<i>Dlg4</i>	0.9998	0.5281	0.9999
		<i>Ina</i>	0.9998	0.9080	0.9999
		<i>Map2</i>	0.9998	0.9898	0.9999
		<i>Mapt</i>	0.9998	0.9510	0.9999
		<i>Nefh</i>	0.9998	0.7970	0.9999
		<i>Nefl</i>	0.9998	0.9357	0.9999
		<i>Nefm</i>	0.9998	0.9084	0.9999
		<i>Nrp1</i>	0.9998	0.8505	0.9999
		<i>Rbfox3</i>	0.9998	0.8978	0.9999
		<i>Syp</i>	0.9998	0.5319	0.9999
		<i>Thy1</i>	0.9998	0.8695	0.9999
		<i>Tubb3</i>	0.9998	0.9984	0.9999
		<i>Uchl1</i>	0.9998	0.9866	0.9999
	Cholinergic neurons	<i>Ache</i>	0.9998	0.9966	0.9999
		<i>Chat</i>	0.0003	0.2561	0.0241
		<i>Slc5a7</i>	0.0004	0.0068	0.0034
	Dopaminergic neurons	<i>Dbh</i>	0.9998	0.9168	0.9999
		<i>Foxa2</i>	NA	NA	0.9999
		<i>Kcnj3</i>	0.9998	0.1830	0.9999

		<i>Lmx1b</i>	NA	0.9587	0.9999
		<i>Nr4a2</i>	0.9998	0.8519	0.9999
		<i>Ppp1r1b</i>	0.9998	0.9686	0.9999
		<i>Slc6a2</i>	0.9998	0.9779	0.9999
		<i>Slc6a3</i>	0.9998	0.8702	0.9999
		<i>Th</i>	0.9998	0.4542	0.9999
	GABAergic neurons	<i>Adora2a</i>	0.9998	0.9714	0.9999
		<i>Calb1</i>	0.9998	0.8686	0.9999
		<i>Calb2</i>	0.9998	0.9586	0.9999
		<i>Gabbr1</i>	0.9998	0.8519	0.9999
		<i>Gabbr2</i>	0.9514	0.9774	0.9999
		<i>Gad1</i>	0.9998	0.9133	0.9999
		<i>Gad2</i>	0.9998	0.8612	0.9999
		<i>Pvalb</i>	0.9998	0.9309	0.9999
		<i>Penk</i>	0.4800	0.8395	0.9999
		<i>Slc6a1</i>	0.9998	0.8702	0.9999
		<i>Slc32a1</i>	0.9998	0.9347	0.9999
		<i>Sst</i>	0.9998	0.8519	0.9999
	Glutamatergic neurons	<i>Gls</i>	0.9998	0.9579	0.9999
		<i>Glul</i>	0.9998	0.9857	0.9999
		<i>Grin1</i>	0.9998	0.9587	0.9999
		<i>Grin2a</i>	0.9998	0.9871	0.9999
		<i>Grin2b</i>	0.9998	0.9083	0.9999
		<i>Slc17a6</i>	0.9998	0.9181	0.9999
		<i>Slc17a7</i>	0.9998	0.5909	0.9999
	Serotonergic neurons	<i>Fev</i>	NA	NA	0.9999
		<i>Slc6a4</i>	NA	0.9391	0.9999
		<i>Tph1</i>	NA	NA	0.9999
		<i>Tph2</i>	0.9998	0.9308	0.9999

Supplementary Table 3. Primers used for qPCR-based analyses

Gene	Primer forward	Primer reverse
<i>Actb</i>	CCCTGAAGTACCCCATTGAAC	CCATGTCGTCCCAGTTGGTAA
<i>Adam10</i>	TGATGGTGTCTTGGTCTGG	CTTGCTTTTCTCACATATTCCCC
<i>Ager</i>	ACCCATCCTACCTTCTCCTG	GAGCGACTATTCCACCTTCAG
<i>Aph1a</i>	CCAGTATGGCCTCCTGATTT	TGATGCTAAGCCCTCATCTG
<i>Aph1b</i>	TTGGTGTCTCTCCTGCTGTC	AGCCTGAACAGCTCTTGGAT
<i>Bace1</i>	TTGTAGGGCTAGGGATGGTC	CCTAACCTGCTGGATGAAT
<i>Ccl2</i>	AAGAGATCAGGGAGTTTGCT	CTGCCTCCATCAACCACTTT
<i>Ccl6</i>	TCTTTATCCTTGTGGCTGTCC	ATGGGATCTGTGTGGCATAAG
<i>Cd14</i>	TTTAACTCTGGCGTAGTCACC	GACCCTCAGAAACCAGGAG
<i>Cd36</i>	GCGACATGATTAATGGCACAG	GATCCGAACACAGCGTAGATAG
<i>Cd68</i>	GTTACTCTCCTGCCATCCTTC	GCAGGGTTATGAGTGACAGTTG
<i>Gfap</i>	GAAAACCGCATCACCATTCC	CTTAATGACCTCACCATCCCG
<i>Ide</i>	CCCGACTTGATAGACATGGTTC	CGGTCAGTTTTCCCTTCAAATG
<i>Ifng</i>	CTTTGGACCCTCTGACTTGAG	TCAATGACTGTGCCGTGG
<i>Il1b</i>	GAAGAAGAGCCCATCCTCTG	TCATCTCGGAGCCTGTAGTG
<i>Il6</i>	AGTCCGGAGAGGAGACTTCA	ATTTCCACGATTTCCAGAG
<i>Itgam</i>	CATCCCATGACCTTCCAAGAG	GTGCTGTAGTCACACTGGTAG
<i>Lrp1</i>	GGGAAGTGATGGGAAGTCTTG	GGATTCATAAGGTTCTCGATGGG
<i>Mme</i>	GATGAGTGGATAAGTGGAGCAG	CAATGAGTTGGATTGCTGAGC
<i>Msr1</i>	GGGAACACTCACAGACACTG	CCCGATCACCTTTAACACCTG
<i>Ncstn</i>	GTA CTGGCAGGATTGTGTGG	ATCTGATGAGTGGCGTTGAG
<i>Psen1</i>	CTGGTTGAAACAGCTCAGGA	TTCTTGGGTACCCTCCTTTG
<i>Psen2</i>	ACGCTGTGTATGATCGTGGT	TGTTAAGCACGGAGTTGAGG
<i>Psenen</i>	GGTTCTTCAGAGAGGCGTTC	GATCACCCAGAAGAGGAAGC
<i>Tlr2</i>	ACA ACTTACCGAAACCTCAGAC	ACCCAGAAAGCATCACATG
<i>Tnf</i>	CTTCTGTCTACTGAACTTCGGG	CAGGCTTGTCCTCGAATTTTG
<i>Trem2</i>	GCTTGGTCATCTCTTTTCTGC	GTTGAGGGCTTGGGACAG

Supplementary Figure 1. Analyses of behavioral and cognitive assessments stratified by sex.

(a) Distance traveled, (b) immobile duration, (c) mobile duration, and (d) movement speed recorded in the open field test (5 male and 5 female 18mo controls; 5 male and 6 female APP 6→18mo; 5 male and 5 female 24mo controls; 5 male and 6 female APP 12→24mo). (e) Latency to fall on the accelerating rotarod (5 male and 5 female 18mo controls; 6 male and 6 female APP 6→18mo; 5 male and 5 female 24mo controls; 5 male and 6 female APP 12→24mo). (f) Latency to fall on the inverted screen test (5 male and 5 female 18mo controls; 5 male and 6 female APP 6→18mo; 5 male and 5 female 24mo controls; 5 male and 6 female APP 12→24mo). (g) Percent time spent freezing during the test session in a contextual fear conditioning paradigm (5 male and 5 female 18mo controls; 5 male and 6 female APP 6→18mo; 5 male and 5 female 24mo controls; 5 male and 6 female APP 12→24mo). (h) Swim speed and (i) escape latency during the training phase of the Morris Water Maze (MWM) (5 male and 5 female 18mo controls; 5 male and 6 female APP 6→18mo; 5 male and 4 female 24mo controls; 5 male and 6 female APP 12→24mo). (j) Time spent in the target quadrant (TQ) vs. the average of all other quadrants (AOQ), and (k) number of platform crossings during the MWM probe trial. The panels (i-k) were analyzed by three-way ANOVA. Individual data points and group means $\pm$ S.E.M. are shown. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 2. Levels of APP and brain amyloid burden stratified by sex.

Quantification of (a) full-length APP and (b) APP C-terminal fragments (APP-CTFs) in 18mo control (5 males and 5 females), APP 6→18mo (5 males and 5 females), 24mo control (5 males and 5 females), and APP 12→24mo (5 males and 5 females). Quantification of (c) BACE

and (d) PS1 C-terminal fragments (PS1-CTFs) in 18mo control (5 males and 5 females), APP 6→18mo (5 males and 6 females), 24mo control (5 males and 5 females), and APP 12→24mo (5 males and 6 females). ELISA-based measurements of (e) A β 40 and (f) A β 42 levels in TBS-fraction (4 male and 4 female 18mo controls; 3 male and 4 female APP 6→18mo; 4 male and 4 female 24mo controls; 4-5 male and 4 female APP 12→24mo), TBS-triton-fraction (4 male and 4 female 18mo controls; 3 male and 4 female APP 6→18mo; 4 male and 5 female 24mo controls; 4-5 male and 4 female APP 12→24mo), and GuHCl-fraction (4 male and 4 female 18mo controls; 3 male and 4 female APP 6→18mo; 4 male and 5 female 24mo controls; 5 male and 4 female APP 12→24mo). (g) A β 40/A β 42 ratio calculated for TBS and TBS + Triton X-100 soluble A β species. (h) A β 40/A β 42 ratio for GuHCl-soluble higher-order A β aggregates. Individual data points and group means $\pm$ S.E.M. are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 3. Gene expression levels of α -, β -, and γ -secretase components measured by qPCR.

mRNA levels of (a) *Adam10*, (b) *Bace1*, (c) *Psen1*, (d) *Psen2*, (e) *Ncstn*, (f) *Psenen*, (g) *Aph1a*, and (h) *Aph1b* were measured in brain tissue from APP 6→18mo (4 males and 4 females), APP 12→24mo (4 males and 4 females), and age-matched control mice (4 male and 4 female 18mo controls; 4 male and 3-4 female 24mo controls). Individual data points as well as mean $\pm$ S.E.M. are shown. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 4. Sex-stratified analyses of gene transcription of receptors and enzymes critical for A β clearance and degradation.

mRNA levels of (a) *Ager*, (b) *Cd14*, (c) *Cd36*, (d) *Lrp1*, (e) *Msr1*, (f) *Tlr2*, (g) *Ide*, and (h) *Mme* were measured in the brains of APP 6→18mo (4 males and 4 females), APP 12→24mo (4

males and 4 females), and age-matched control animals (4 male and 4 female 18mo controls; 4 male and 3-4 female 24mo controls). Individual data points and group means $\pm$ S.E.M. are shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure 5. mRNA levels of inflammatory cytokines and chemokines stratified by sex.

mRNA levels of (a) *Ifng*, (b) *Il1b*, (c) *Il6*, (d) *Tnf*, (e) *Ccl2*, and (f) *Ccl6* were measured in the brains of APP 6→18mo (4 males and 4 females), APP 12→24mo (3-4 males and 4 females), and age-matched control animals (3-4 male and 4 female 18mo controls; 4 male and 2-4 female 24mo controls). Individual data points and group means $\pm$ S.E.M. are shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 6. Abundance of autophagy marker proteins stratified by sex.

Levels of (a) LC3A-II/LC3A-I ratio, (b) total LC3A, (c) LC3B-II/LC3B-I ratio, (d) total LC3B, (e) ATG3, (f) ATG5, (g) ATG7, (h) ATG12, (i) Beclin-1, and (j) SQSTM1/p62 were determined by western blot. Sample size was 5 male and 5 female 18mo controls, 4-5 male and 6 female APP 6→18mo, 4-5 male and 5 female 24mo controls, and 5 male and 5-6 female APP 12→24mo. Individual data points and group means $\pm$ S.E.M. are presented. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 7. Analyses of canonical pathways, diseases and functions, and upstream regulators.

(a) Canonical pathways, (b) disease and function annotations, and (c) upstream regulators associated with differentially expressed genes were identified by comparing brain transcriptomic profiles between APP 6→18mo (2 males and 2 females), APP 12→24mo (2 males and 2 females), and age-matched controls (2 male and 1 female 18-month-old controls; 2 male and 2 female 24-month-old controls) using Ingenuity Pathway Analysis (IPA). A false discovery rate (FDR) of 0.05 was applied. Predicted pathway activation is shown in red, while predicted inhibition is indicated in blue.

Supplementary Figure 8. Gene expression levels of astrocytic and microglial lineage markers determined by qPCR.

mRNA levels of (a) *Gfap*, (b) *Itgam*, (c) *Trem2*, and (d) *Cd68* were quantified in brain tissue from APP 6→18mo (4 males and 4 females), APP 12→24mo (4 males and 4 females), and age-matched control animals (4 male and 4 female 18mo controls; 4 male and 3-4 female 24mo controls). Individual data points as well as means $\pm$ S.E.M. are presented. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 9. Protein abundance of astrocytic and microglial lineage markers measured by western blot.

(a) Representative western blot images and quantification of (b) GFAP, (c) CD11b, (d) CD68, (e) IBA1, and (f) TREM2 in 18mo control (5 males and 5 females), APP 6→18mo (5 males and 6 females), 24mo control (5 males and 5 females), and APP 12→24mo (5 males and 6 females) are shown. Individual data points as well as means $\pm$ S.E.M. are presented. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Supplementary Figure 10. Sex-stratified analyses of cholinergic markers at the transcriptional and protein levels.

mRNA levels of (a) *Chat*, (b) *Slc5a7*, and (c) *Slc18a3* were measured by qPCR in 18mo control (4 males and 4 females), APP 6→18mo (4 males and 4 females), 24mo control (4 males and 3-4 females), and APP 12→24mo (4 males and 3-4 females). Protein levels of (d) ChAT and (e) SLC5A7 were quantified by western blot in 5 male and 5 female 18mo controls, 5 male and 5-6 female APP 6→18mo, 4-5 male and 5 female 24mo controls, and 4-5 male and 6 female

APP 12→24mo. Individual data points and group means $\pm$ S.E.M. are presented. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure 11. Abundance of synaptic marker proteins was reduced with age but was not affected by APP expression.

(a) Representative western blot images are shown. Protein levels of (b) PSD95 and (c) synaptophysin (SYP) were quantified by western blot in 18mo control (5 males and 5 females), APP 6→18mo (5 males and 6 females), 24mo control (4-5 males and 5 females), and APP 12→24mo (5 males and 6 females). Individual data points as well as means $\pm$ S.E.M. are presented. * $p<0.05$, ** $p<0.01$.

Supplementary Data 1. Differentially expressed genes identified by whole-brain RNA sequencing.

This file contains genes differentially expressed in association with sex, age, mutant APP expression, and the age $\times$ APP interaction. Analyses were performed by comparing brain transcriptomes of APP 6→18mo (2 males and 2 females), APP 12→24mo (2 males and 2 females), and age-matched control mice (2 male and 1 female 18-month-old controls; 2 male and 2 female 24-month-old controls). A false discovery rate (FDR) threshold of 0.05 was applied.

Supplementary Data 2. Canonical pathway, disease/function, and upstream regulator analyses by Ingenuity Pathway Analysis.

This file summarizes canonical pathways, disease and biological functions, and upstream regulators identified through Ingenuity Pathway Analysis (IPA) of differentially expressed genes in association with age, mutant APP expression, and the age $\times$ APP interaction. Analyses were based on comparisons of brain transcriptomic profiles between APP 6→18mo (2 males and 2 females), APP 12→24mo (2 males and 2 females), and age-matched control mice (2 male and 1 female 18-month-old controls; 2 male and 2 female 24-month-old controls). A false discovery rate (FDR) threshold of 0.05 was applied.