

1 **Alterations in endocytic protein expression with increasing age in the**  
2 **transgenic APP<sub>695</sub> V717I London mouse model of amyloid pathology –**  
3 **implications for Alzheimer’s disease.**

4

5 **Running head:** Altered endocytic protein expression with age

6

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10

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12 None of the authors have any conflicts of interest.

13

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16 Society in the U.K.. MA was supported by Bristol Research into Alzheimer's and  
17 Care of Elderly (BRACE) in the U.K..

18

#### 19 **Authors' contributions**

20 EJK, RST, MHE and MG conceived the study and designed the experiments.

21 MHE bred the mice. MA and JSB performed all the experiments. MA analysed  
22 the data and EJK assisted with data interpretation. EJK and MA wrote the

23 manuscript. All authors read and commented on the text and approved the final

24 version of the manuscript.

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1 **Abstract**

2 A major risk factor for the development of Alzheimer's disease is increasing age  
3 but the reason behind this association has not been identified. It is thought that  
4 the changes in endocytosis seen in Alzheimer's disease patients are causal for  
5 this condition. Thus we hypothesised that the increased risk of developing  
6 Alzheimer's disease associated with ageing may be due to changes in  
7 endocytosis. We investigated using Western blotting whether the expression of  
8 endocytic proteins involved in clathrin-mediated and clathrin-independent  
9 endocytosis are altered by increasing age in a mouse model of amyloid  
10 pathology. We used mice transgenic for human amyloid precursor protein  
11 containing the V717I London mutation. We compared London mutation mice  
12 with age-matched wild-type controls at three ages, 3, 9 and 18 months,  
13 representing different stages in the development of pathology in this model.  
14 Having verified that the London mutation mice over-expressed amyloid  
15 precursor protein and  $\beta$ -amyloid, we found that the expression of the smallest  
16 isoform of PICALM, a key protein involved in the regulation of clathrin-coated pit  
17 formation, was significantly increased in wild-type mice but decreased in  
18 London mutation mice with age. PICALM levels in wild-type 18-month mice and  
19 clathrin levels in wild-type 9-month mice were significantly higher than those in  
20 London mutation mice of the same ages. The expression of caveolin-1, involved  
21 in clathrin-independent endocytosis, was significantly increased with age in all  
22 mice. Our results suggest that endocytic processes could be altered by the  
23 ageing process and such changes could partly explain the association between  
24 ageing and Alzheimer's disease.

1 **Keywords**

- 2 Alzheimer's disease;  $\beta$ -amyloid; amyloid precursor protein; caveolin; clathrin-  
3 independent endocytosis; clathrin-mediated endocytosis; endocytosis; London  
4 mutation; mouse model; PICALM; V717I

## 1 **Introduction**

2 Dementia currently affects about 47.5 million people worldwide [1] with  
3 Alzheimer's disease (AD) being the most common cause [1,2]. The major risk  
4 factor for developing AD is increasing age, with about 11% of people aged 65  
5 and over having the disease, rising to 32% over the age of 85 [2]. It is estimated  
6 that by 2050 there will be over 115 million people in the world with AD [3].  
7 However, few studies have considered how ageing may contribute to the  
8 aetiology of the disease.

9  
10 Alterations in endocytosis represent a potential mechanism which could  
11 underlie the association of AD with age. Changes in endocytosis were first  
12 identified in cases of AD twenty years ago [4]. Since 2009, a number of  
13 Genome Wide Association Studies have described polymorphisms in genes  
14 associated with a small increased risk of developing AD and at least three of the  
15 proteins encoded by these genes, *PICALM*, *BIN1* and *SORL1*, are involved in  
16 endocytosis [5], emphasising the importance of this pathway in AD. Amyloid  
17 precursor protein (APP), the source of  $\beta$ -amyloid ( $A\beta$ ), a key protein involved in  
18 the pathology of AD, is transported via the secretory pathway to the cell surface  
19 and is then internalised by endocytosis. Most amyloidogenic processing occurs  
20 only after this event, within the endocytic/lysosomal system [6,7]. Endocytosis is  
21 thus central to the production of  $A\beta$ .

22  
23 Clathrin-mediated endocytosis (CME) involves many different proteins including  
24 the scission protein dynamin and regulatory accessory or adaptor proteins such

1 as AP180, PICALM, amphiphysin I and BIN1 [8]. Changes in CME have been  
2 seen in AD and associated models of amyloid pathology. Early endocytic  
3 changes, as evidenced by an increase in the number and size of Rab5-positive  
4 endosomes, are present in Down Syndrome and AD brains [9]. Importantly,  
5 inhibition of both CME in vivo in APP transgenic mice and dynamin-dependent  
6 endocytosis in vitro lowered A $\beta$  levels [10,11], while up-regulation of  
7 endocytosis increased APP metabolism to sAPP $\beta$  and  $\beta$ CTF and increased A $\beta$   
8 secretion [7].

9  
10 Lipid rafts in the plasma membrane are also important for modulating A $\beta$   
11 production [12,13]. Caveolae are a type of lipid raft enriched with caveolins-1-3  
12 and are associated with processes including clathrin-independent endocytosis  
13 (CIE) [14]. Flotillin-1 and -2, also found in lipid rafts, non-caveolar lipid raft  
14 microdomains in neurones that may also be implicated in endocytosis [15,16].  
15 Alterations in caveolins have been associated with AD as the expression of  
16 caveolin-1 is elevated in the hippocampus in AD compared to non-AD brains  
17 [17].

18  
19 We have previously considered the importance of changes in endocytosis with  
20 ageing for AD pathophysiology by examining the expression of several CME  
21 and CIE-related endocytic proteins in the cortex of aged transgenic (Tg) Tg2576  
22 mice expressing the Swedish mutation of human APP at the  $\beta$ -secretase  
23 cleavage site [18]. Using 22 month-old male mice, we found significantly higher  
24 levels of clathrin heavy chain (CHC), dynamin II and PICALM compared to wild-

1 type (WT) mice but no changes in proteins involved in CIE [18]. However, we  
2 did not compare different ages. Therefore we have now investigated how  
3 ageing and the presence of amyloid pathology affect the expression of a range  
4 of proteins involved in CME and CIE. We have used the London V717I mouse,  
5 a well-characterised model of amyloid pathology that overexpresses human  
6 APP<sub>695</sub> with a mutation at V717I [19]. In contrast to our earlier data from the  
7 Tg2576 mouse [18], we did not see any changes in clathrin expression with age  
8 or between genotypes but we did identify an increase in the expression of  
9 caveolin-1 with age in WT and Tg mice. Furthermore, interestingly we saw an  
10 increase in the expression of an isoform of PICALM in WT mice but a decrease  
11 in Tg mice with age.

12

### 13 **Methods**

#### 14 *Materials*

15 All chemicals and reagents were purchased from Sigma-Aldrich, Poole, U.K. or  
16 Fisher Scientific, Leicester, U.K unless specified. Antibodies used in Western  
17 blotting were: anti N-APP, 22C11 (Millipore, Watford, U.K.); anti-clathrin heavy  
18 chain (CHC, Clone 23), anti-caveolin-1, anti-caveolin-2 (Clone 65), anti-flotillin-1  
19 (Clone 18), (BD Biosciences, Oxford, UK); anti-GAPDH (Sigma-Aldrich); anti-  
20 BIN1 (Santa Cruz, Wembley, U.K.); anti-PICALM, anti-flotillin-2 (Novus  
21 Biologicals, Littleton, CO, USA); anti-dynamin-1 (Abcam, Cambridge, MA).

22

23

24



1 *Mice*

2 Tg mice carrying the London V717I mutation in human APP [19] were  
3 maintained on the in-bred C57Bl/6 background. All work described here  
4 complied with the guidelines for the care and use of laboratory animals  
5 according to the Animals (Scientific Procedures) Act 1986 and in accordance  
6 with Home Office (U.K.) regulations and European Union directive 2010/63/EU.

7

8 *Protein Extraction*

9 Soluble and insoluble proteins were extracted from the total cortices of male 3,  
10 9 and 18-month old London Tg mice and WT aged-matched littermates  
11 following the method of Rees et al. [18,20]. Total protein concentration was  
12 determined with the BCA Protein Assay Kit (Thermo Scientific, Waltham, USA).

13

14 *Western Blotting*

15 Western Blotting was performed using standard methods. Briefly, after protein  
16 analysis, 10µg of all samples were resolved on 10% polyacrylamide gels, and  
17 detected with the relevant antibody as previously described [18].

18

19 *ELISA*

20 Soluble and insoluble human Aβ40 and 42 were detected by ELISA as  
21 previously described [18].

22

23

24

1 *Statistical analyses*

2 The Western blot shown for each protein was quantified using Image J  
3 (www.imagej.nih.gov). All protein bands were expressed as the relative density  
4 of WT cortex sample 1 and then normalised for relative GAPDH levels. ELISA  
5 data were expressed as ng A $\beta$ /mg total protein. The blotting data were analysed  
6 by one-way ANOVA followed by Tukey's post-hoc tests or by unpaired  
7 Student's t-tests to determine if protein levels differed significantly between  
8 ages or between Tg and age-matched WT mice, respectively. ELISA data were  
9 analysed using Kruskal-Wallis followed by Dunn's multiple comparisons test  
10 (soluble A $\beta$ ) or one-way ANOVA followed by Fisher's LSD test (insoluble A $\beta$ 40).  
11 Where necessary, data were transformed to fit the assumptions of normality.

12

13 **Results**

14 *Expression of APP, A $\beta$ 40 and A $\beta$ 42 in the Tg and WT mice*

15 As expected the expression of APP was significantly increased by about 3-fold  
16 in the cortex of 18-month Tg mice compared to WT mice ( $p < 0.05$ ) (Fig. 1A). The  
17 levels of APP were not altered by ageing in either WT or Tg mice (see Figure  
18 Supplemental Digital Content 1). Soluble and insoluble A $\beta$ 40 and soluble A $\beta$ 42  
19 levels from the overexpressed human APP were all significantly increased in  
20 18-month Tg mice compared to younger mice ( $p < 0.05$ ) (Fig. 2A, B).

21

22 *Levels of proteins involved in CME are altered by ageing and APP genotype*

23 No significant differences in the levels of clathrin heavy chain (CHC) with  
24 increasing age were detected in either WT or Tg mice (Fig. 1B,C). In contrast,

1 when the level of clathrin was compared between WT and Tg mice brains, a  
2 significant decrease was seen in 9-month Tg mice,  $0.3 \pm 0.02$  compared to  $0.8$   
3  $\pm 0.1$  in WT mice ( $p < 0.001$ , OD ratio relative to GAPDH). There were no  
4 significant changes in the levels of clathrin between WT and Tg mice aged 3  
5 and 18 months (see Table Supplemental Digital Content 2).

6 The levels of dynamin I were not significantly altered by ageing in either WT or  
7 Tg mice (see Figure Supplemental Digital Content 1). Furthermore, no changes  
8 in dynamin were observed between WT and Tg mice of the same age (see  
9 Table Supplemental Digital Content 2).

10 At least 6 isoforms of *Mus musculus* PICALM have been found with predicted  
11 molecular masses ranging from approximately 64 to 72 kDa (NCBI RefSeq). We  
12 identified PICALM as 3 distinct bands at 72, 68, and 62 kDa (Fig. 1D-F). The  
13 largest bands (bands 1 and 2) were analysed together as they were not fully  
14 resolved. There was no detectable change in the levels of bands 1 and 2 with  
15 ageing in either WT or Tg mice (Fig. 1E,F). There were also no changes in the  
16 expression of bands 1 and 2 between WT and Tg mice aged 3, 9 and 18  
17 months (Fig. 1D, see Table Supplemental Digital Content 2). However,  
18 expression of the smallest band of PICALM (band 3) was significantly increased  
19 in 18-month WT mice compared to 9-month mice (Fig. 1E) but was significantly  
20 decreased in 18-month Tg mice compared to 3-month mice (Fig. 1F).  
21 Furthermore, the levels of band 3 were significantly reduced by approximately 6  
22 times in 18-month Tg mice when compared to WT mice (Fig. 1D). In contrast,

1 there were no changes in the expression of band 3 between WT and Tg mice  
2 aged 3 and 9 months (see Table Supplemental Digital Content 2).

3 At least 15 different isoforms of bridging integrator 1 (Bin-1) have been  
4 identified. Here, two bands were observed for Bin-1 (see Figure Supplemental  
5 Digital Content 1). The levels of Bin-1 were not significantly altered in Tg mice  
6 compared to WT mice at any age point (see Table Supplemental Digital Content  
7 2). Similarly, no significant changes were detected in the levels of Bin-1 with  
8 ageing in either WT or Tg mice (see Figure Supplemental Digital Content 1).

9

#### 10 *Levels of proteins involved in CIE are altered by ageing and genotype*

11 The levels of caveolin-1 were significantly higher in both 9- and -18 month WT  
12 and Tg mice compared to 3-month mice (Fig. 2A,B). However, the levels of  
13 caveolin-1 were not altered between 3- and 9-month old WT and Tg mice (see  
14 Table Supplemental Digital Content 2) but were significantly decreased by  
15 approximately 1.4 times in 18-month Tg compared to WT mice (Fig. 2C).

16 Caveolin-2 expression in WT and Tg mice was not altered by age or genotype  
17 (see Figure and Table Supplemental Digital Content 1 and 2). Neither flotillin-1  
18 nor flotillin-2 in WT and Tg mice were affected by age or genotype (see Figure  
19 and Table Supplemental Digital Content 1 and 2).

20

#### 21 **Discussion**

22 The results presented here show that both ageing and genotype affected the  
23 expression of endocytic proteins in the cortex of WT and Tg V717I London

1 mutation mice. The data obtained for APP expression confirmed firstly that the  
2 Tg London mutation mice over-expressed APP compared to the WT mice and  
3 secondly showed no change in APP expression with age, as expected from  
4 other studies [21]. This overexpression of APP led to the expected increase in  
5 human A $\beta$ 40 and 42 in the 18-month Tg mice.  
6  
7 Interestingly, although there is much evidence, reviewed above, to show that  
8 CME is affected in AD and implicated in the pathogenesis of the disease, we  
9 saw limited changes in clathrin itself with a decrease only in 9-month Tg mice  
10 compared to WT mice. We know that cognitive deficits start to appear around 6-  
11 9 months of age in these mice which could be linked to the change in clathrin  
12 seen here (unpublished data). Interestingly, the decrease in clathrin precedes  
13 the increase in A $\beta$  in these mice so a small change in endocytosis in earlier life  
14 might be linked to the subsequent rise in A $\beta$  levels in these mice. However, in  
15 the cortex of the London mutation mice neither age nor the presence of amyloid  
16 pathology appeared to have a large effect on CME as determined by the  
17 expression levels of clathrin. Support is provided for this conclusion by the data  
18 for dynamin-1, PICALM bands 1 and 2 and Bin-1 where no changes in  
19 expression were seen. This is particularly significant for dynamin-1, crucial for  
20 CME to occur but also for many forms of CIE. The data for band 3 for PICALM,  
21 however, do not fit with this conclusion as its expression was differentially  
22 affected by both age and genotype. Currently, the function of the different  
23 PICALM isoforms is not understood and we have previously shown that at least  
24 human isoforms 1 and 2 are required for PICALM to affect functional

1 endocytosis in the H4 cell line [22]. The most likely explanation for these  
2 findings lies in another role of PICALM, in addition to its involvement in CME.  
3 More specifically, PICALM controls the endocytosis of R-SNAREs (Soluble NSF  
4 Attachment Protein Receptors) necessary for the fusion of endocytic vesicles  
5 with endosomes or the plasma membrane [23].  
6  
7 There does appear to be some involvement of ageing and genotype in CIE as  
8 the expression of caveolin-1 was increased in both genotypes with age but to a  
9 larger extent in the WT mice. Furthermore, this increase in caveolin-1 preceded  
10 the increase in A $\beta$ 40 and 42 in the Tg mice supporting the data with clathrin and  
11 possibly suggesting that changes in endocytosis could be linked to subsequent  
12 increases in A $\beta$  accumulation. Another study also found an increase in caveolin-  
13 1 with age in WT mice [17]. This is an interesting result as loss of caveolin-1 is  
14 associated with accelerated aging and neurodegeneration in mice [24]. These  
15 data support those for caveolin-1 expression in the human brain where higher  
16 expression was seen in the hippocampus from AD brains compared to non-AD  
17 brains [17]. We also detected a significant rise in caveolin-1 expression in  
18 human AD frontal cortex compared to age-matched controls (unpublished data).  
19 Another study found no changes in caveolin-1 in human cortex comparing AD  
20 and control individuals [25] but the effect of age was not considered. The other  
21 CIE proteins examined here, caveolin-2, flotillin-1 and -2 were not affected by  
22 ageing or genotype suggesting that any effect of these factors on CIE in mice is  
23 not wide-spread.  
24

1 The results we have obtained here for various endocytic proteins in the V717I  
2 London mutation mice contrast with those we obtained for the same proteins in  
3 old Tg2576 mice [18]. One possible explanation for these differences lies in the  
4 different mutations affecting the  $\beta$ -secretase (Tg2575) and  $\gamma$ -secretase (V717I)  
5 cleavage sites in APP leading to higher levels of APP and 10 to over 1400-fold  
6 increases in A $\beta$  in the Tg2576 mice [18]. In addition, the different genetic  
7 backgrounds of the two mice strains probably also affect other proteins and  
8 biochemical pathways such as those involved in endocytosis.

9

10 In conclusion, we have shown that proteins involved in both CME and CIE are  
11 affected by ageing and also by the presence of amyloid pathology in mice. Our  
12 data provide support for the idea that changes in endocytosis are involved in the  
13 pathogenesis of AD.

14

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5



## 1   **References**

- 2   [1]    WHO. Factsheet on dementia.  
3        <http://www.who.int/mediacentre/factsheets/fs362/en/>, accessed 18/11/16  
4        2016.
- 5   [2]    Alzheimer's Association. 2016 Alzheimer's disease facts and figures.  
6        Alzheimer's & Dementia. 2016;12:459-509
- 7   [3]    Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global  
8        prevalence of dementia: A systematic review and metaanalysis.  
9        Alzheimer's & Dementia. 2013;9:63-75
- 10 [4]    Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, Nixon RA.  
11        Abnormalities of the endosomal-lysosomal system in Alzheimer's  
12        disease: relationship to disease pathogenesis. Adv Exp Med Biol.  
13        1996;389:271-280
- 14 [5]    Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez  
15        C *et al.* Meta-analysis of 74,046 individuals identifies 11 new  
16        susceptibility loci for Alzheimer's disease. Nature Genetics.  
17        2013;45:1452-1458
- 18 [6]    Koo EH, Squazzo SL, Selkoe DJ, Koo CH. Trafficking of cell-surface  
19        amyloid beta-protein precursor. I. Secretion, endocytosis and recycling  
20        as detected by labeled monoclonal antibody. J Cell Sci. 1996;109:991-  
21        998
- 22 [7]    Grbovic OM, Mathews PM, Jiang Y, Schmidt SD, Dinakar R, Summers-  
23        Terio NB *et al.* Rab5-stimulated up-regulation of the endocytic pathway  
24        increases intracellular beta-cleaved amyloid precursor protein carboxyl-

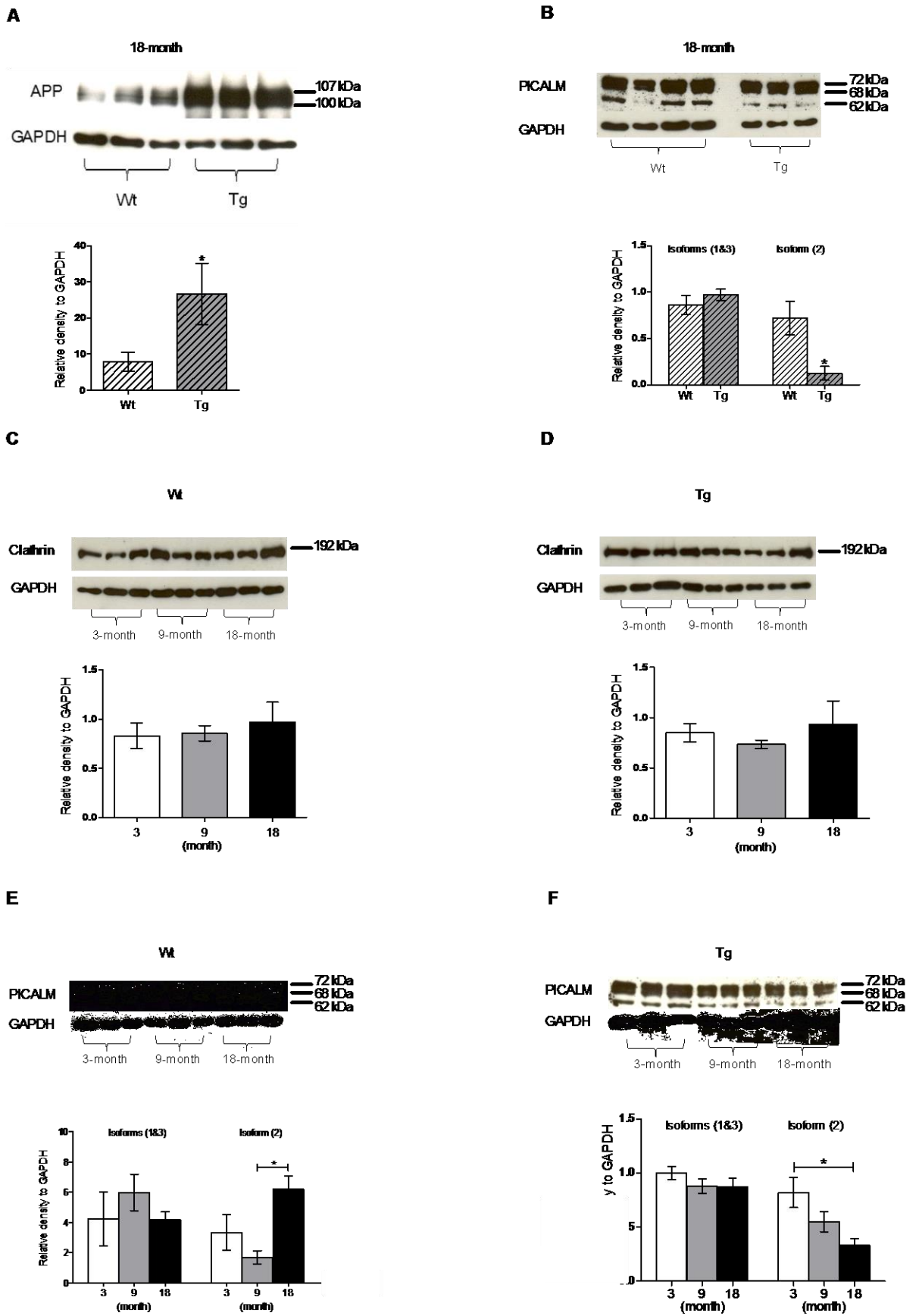
- 1 terminal fragment levels and Abeta production. J B Chem.  
2 2003;278:31261-31268
- 3 [8] Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev  
4 Biochem. 2009;78:857-902
- 5 [9] Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT,  
6 Nixon RA. Endocytic pathway abnormalities precede amyloid beta  
7 deposition in sporadic Alzheimer's disease and Down syndrome:  
8 differential effects of APOE genotype and presenilin mutations. Am J  
9 Path. 2000;157:277-286
- 10 [10] Carey RM, Balcz BA, Lopez-Coviella I, Slack BE. Inhibition of dynamin-  
11 dependent endocytosis increases shedding of the amyloid precursor  
12 protein ectodomain and reduces generation of amyloid beta protein.  
13 BMC Cell Biol. 2005;6:30
- 14 [11] Cirrito JR, Kang JE, Lee J, Stewart FR, Verges DK, Silverio LM *et al.*  
15 Endocytosis is required for synaptic activity-dependent release of  
16 amyloid-beta in vivo. Neuron. 2008;58:42-51
- 17 [12] Cordy JM, Hooper NM, Turner AJ. The involvement of lipid rafts in  
18 Alzheimer's disease. Mol Mem Biol. 2006;23:111-122
- 19 [13] Ehehalt R, Keller P, Haass C, Thiele C, Simons K. Amyloidogenic  
20 processing of the Alzheimer beta-amyloid precursor protein depends on  
21 lipid rafts. J Cell Biol. 2003;160:113-123
- 22 [14] Thomas CM, Smart EJ. Caveolae structure and function. J Cell Mol Med.  
23 2008;12:796-809

- 1 [15] Schneider A, Rajendran L, Honsho M, Gralle M, Donnert G, Wouters F *et*  
2 *al.* Flotillin-dependent clustering of the amyloid precursor protein  
3 regulates its endocytosis and amyloidogenic processing in neurons. *J*  
4 *Neurosci.* 2008;28:2874-2882
- 5 [16] Otto GP, Nichols BJ. The roles of flotillin microdomains--endocytosis and  
6 beyond. *J Cell Sci.* 2011;124:3933-3940
- 7 [17] Gaudreault SB, Dea D, Poirier J. Increased caveolin-1 expression in  
8 Alzheimer's disease brain. *Neurobiol Aging.* 2004;25:753-759
- 9 [18] Thomas RS, Lelos MJ, Good MA, Kidd EJ. Clathrin-mediated endocytic  
10 proteins are upregulated in the cortex of the Tg2576 mouse model of  
11 Alzheimer's disease-like amyloid pathology. *BBRC.* 2011;415:656-661
- 12 [19] Moechars D, Dewachter I, Lorent K, Reverse D, Baekelandt V, Naidu A  
13 *et al.* Early phenotypic changes in transgenic mice that overexpress  
14 different mutants of amyloid precursor protein in brain. *J Biol Chem.*  
15 1999;274:6483-6492
- 16 [20] Rees T, Hammond PI, Soreq H, Younkin S, Brimijoin S.  
17 Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex.  
18 *Neurobiol Aging.* 2003;24:777-787
- 19 [21] Fukumoto H, Rosene DL, Moss MB, Raju S, Hyman BT, Irizarry MC.  $\beta$ -  
20 secretase activity increases with aging in human, monkey, and mouse  
21 brain. *Am J Path.* 2004;164:719-725
- 22 [22] Thomas RS, Henson A, Gerrish A, Jones L, Williams J, Kidd EJ.  
23 Decreasing the expression of PICALM reduces endocytosis and the

- 1 activity of beta-secretase: implications for Alzheimer's disease. BMC  
2 Neuroscience 2016;17:50  
3
- 4 [23] Miller SE, Sahlender DA, Graham SC, Honing S, Robinson MS, Peden  
5 AA *et al.* The molecular basis for the endocytosis of small R-SNAREs by  
6 the clathrin adaptor CALM. Cell. 2011;147:1118-1131
- 7 [24] Head BP, Peart JN, Panneerselvam M, Yokoyama T, Pearn ML,  
8 Niesman IR *et al.* Loss of caveolin-1 accelerates neurodegeneration and  
9 aging. PloS One. 2010;5:e15697
- 10 [25] Van Helmond Z, Miners J, Bednall E, Chalmers K, Zhang Y, Wilcock G *et*  
11 *al.* Caveolin-1 and-2 and their relationship to cerebral amyloid angiopathy  
12 in Alzheimer's disease. Neuropath Applied Neurobiol. 2007;33:317-327  
13

Figure 1

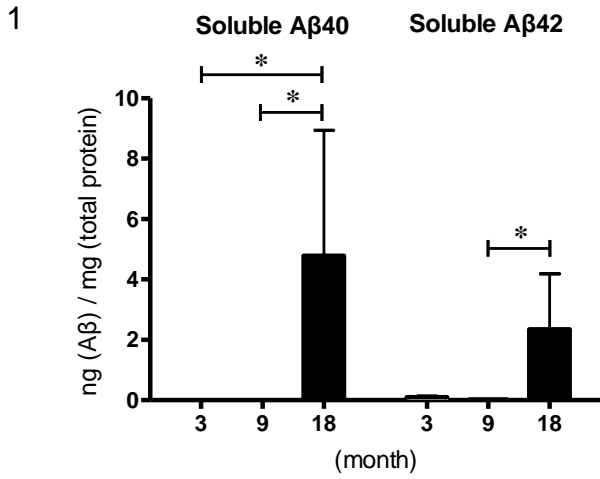
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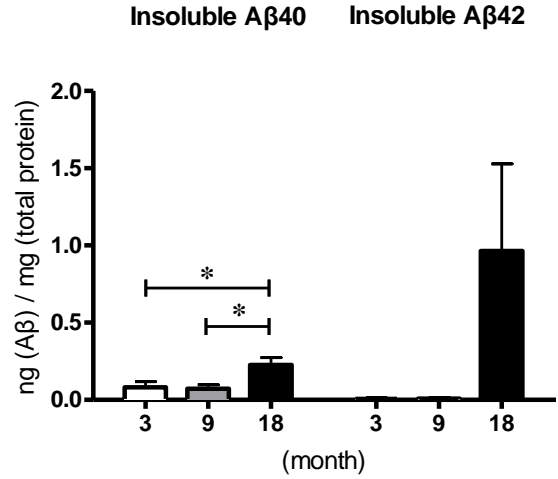
1 **Figure 1.** Comparison of APP, clathrin heavy chain, and 3 bands for PICALM in  
2 the cortex of male WT and Tg mice aged 3, 9 and 18 months. Each section  
3 shows an immunoblot and densitometric analysis of the immunoblot. (A)  
4 Comparison of APP expression in 18 month WT and Tg mice; comparison of  
5 clathrin expression in (B) WT mice and (C) Tg mice aged 3, 9 and 18 months;  
6 comparison of PICALM bands 1&2 and 3 between (D) 18 month WT and Tg  
7 mice and in (E) WT mice and (F) Tg mice aged 3, 9 and 18 months. Levels of  
8 APP were significantly increased in 18 month Tg mice compared to WT mice.  
9 PICALM band 3 expression was significantly decreased in 18 month Tg mice  
10 compared to WT mice and in 18 month Tg mice compared to 3 and 9 month Tg  
11 mice but was increased in 18 month WT mice compared to 3 and 9 month WT  
12 mice. Data are represented as mean  $\pm$  S.E.M. \* $p < 0.05$ , one-way ANOVA  
13 followed by Tukey's post-hoc tests or unpaired Student's t-tests.  $n=3-4$  mice for  
14 each age group.  
15  
16  
17

**Figure 2**

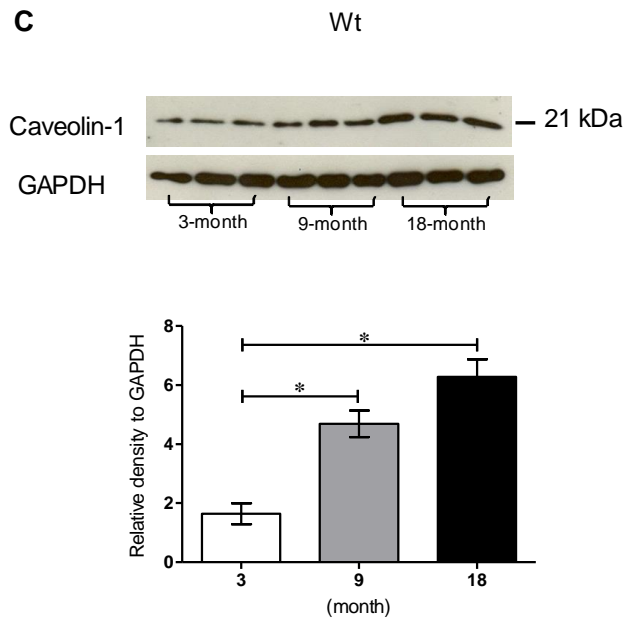
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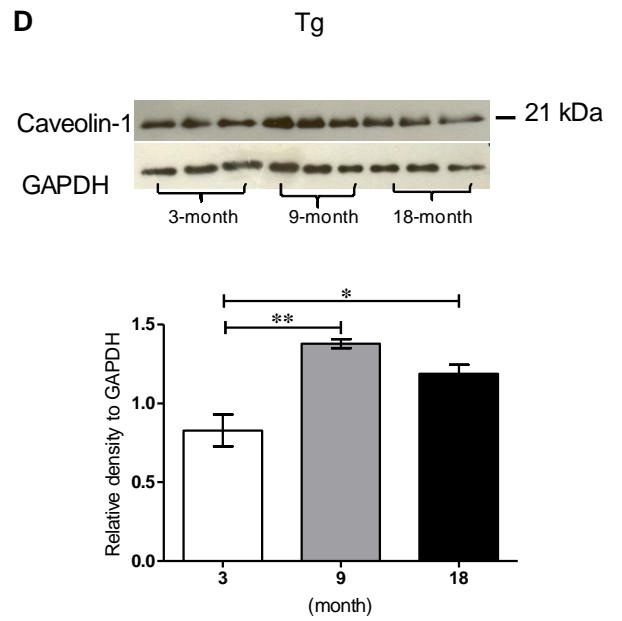
**B**



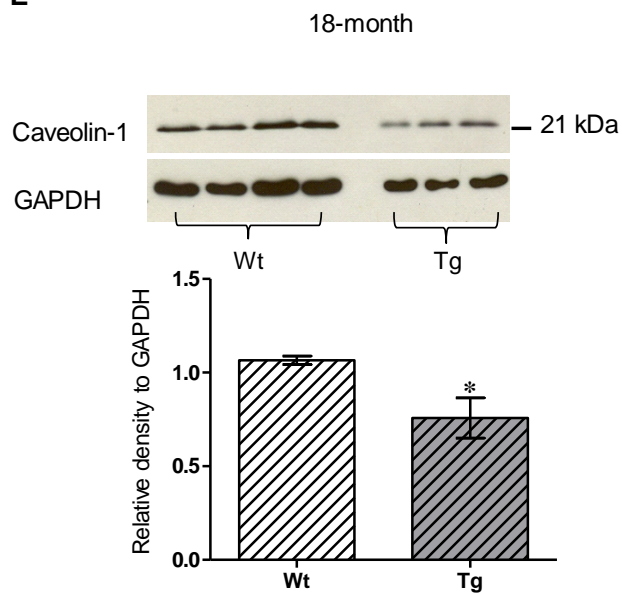
**C**



**D**



**E**



1 **Figure 2.** Comparison of A $\beta$ 40, A $\beta$ 42 and caveolin-1 in the cortex of male WT  
2 and Tg mice aged 3, 9 and 18 months. The caveolin-1 sections each show an  
3 immunoblot and densitometric analysis of the immunoblot. Comparison of  
4 soluble (A) and insoluble (B) A $\beta$ 40 and 42 in Tg mice aged 3, 9 and 18 months;  
5 comparison of caveolin-1 expression in (C) WT mice and (D) Tg mice aged 3, 9  
6 and 18 months; (E) comparison of caveolin-1 expression in 18 month WT and  
7 Tg mice; Soluble and insoluble A $\beta$ 40 was significantly increased in 18 month Tg  
8 mice compared to 3 and 9 month mice while soluble A $\beta$ 42 was significantly  
9 increased in 18 month Tg mice compared to 9 month mice. Levels of caveolin-1  
10 were significantly increased in 9 and 18 month WT and Tg mice compared to  
11 the corresponding 3 month mice. Caveolin-1 expression was significantly  
12 decreased in 18 month Tg mice compared to WT mice. Data are represented as  
13 mean  $\pm$  S.E.M. \* $p$ < 0.05, \*\* $p$ <0.01, ELISAs Kruskal-Wallis followed by Dunn's  
14 multiple comparisons test (soluble A $\beta$ ) or one-way ANOVA followed by Fisher's  
15 LSD test (insoluble A $\beta$ 40); Western blots one-way ANOVA followed by Tukey's  
16 post-hoc tests or unpaired Student's t-tests.  $n$ =3-4 mice for each age group.  
17



1

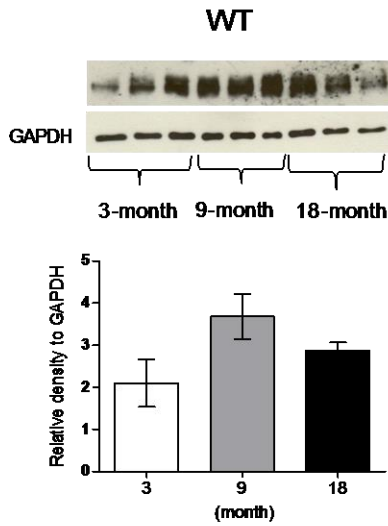
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## Supplemental Digital Content 1

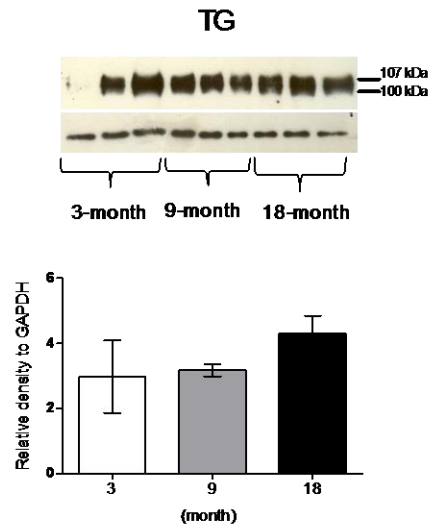
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### APP

A

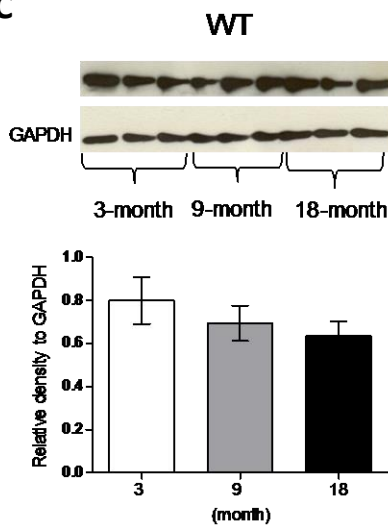


B

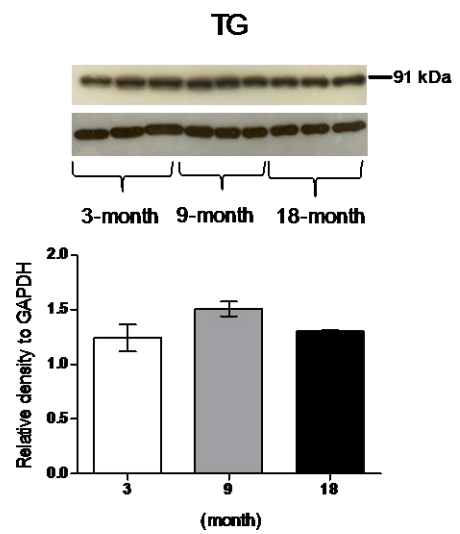


### Dynamin-1

C

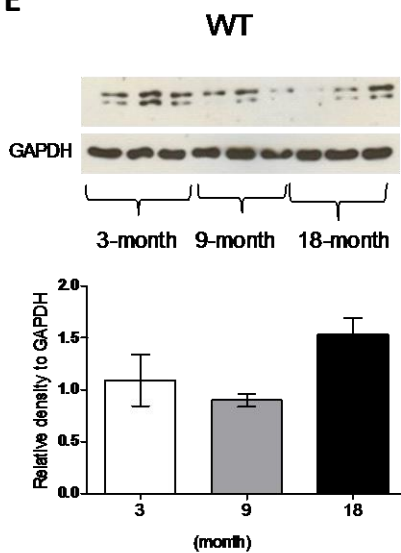


D

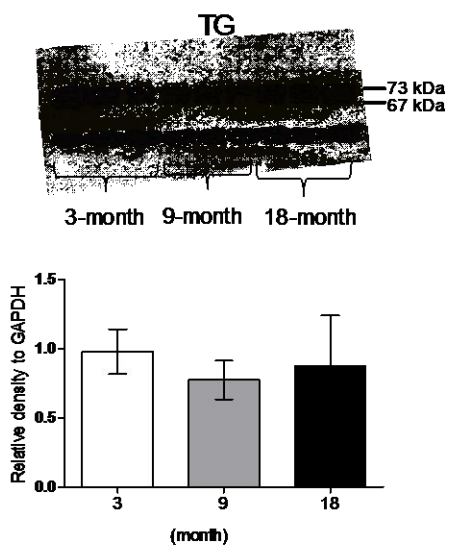


### Bin-1

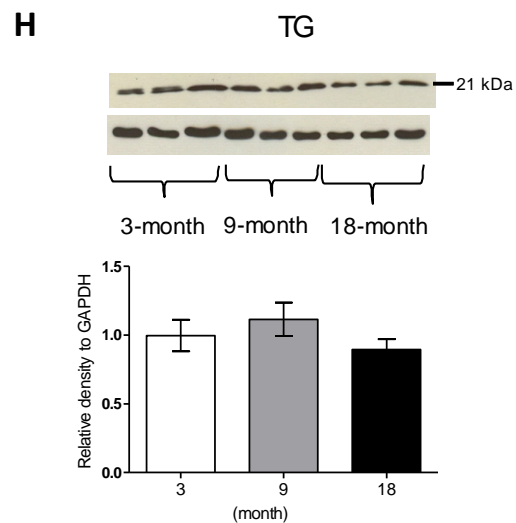
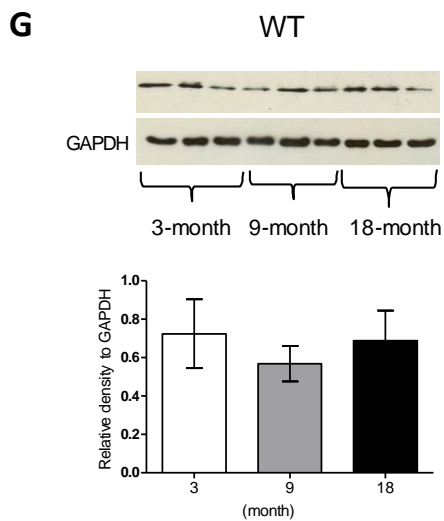
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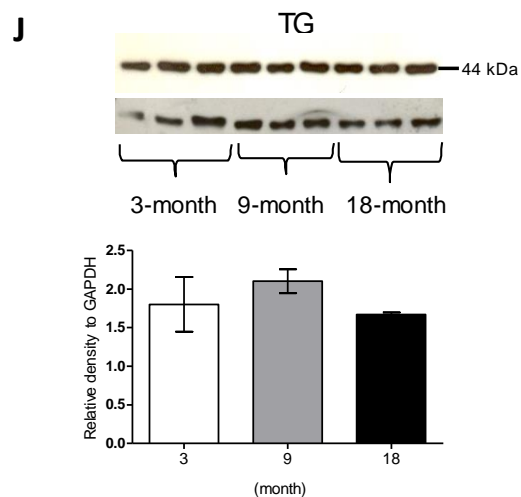
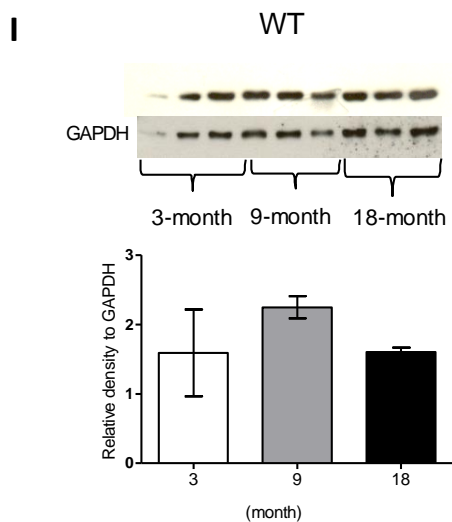
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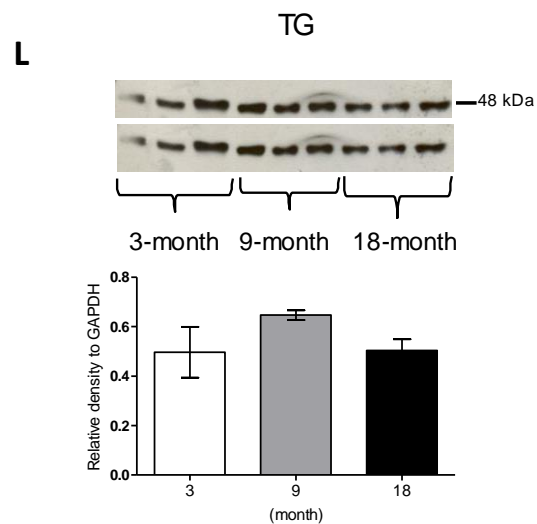
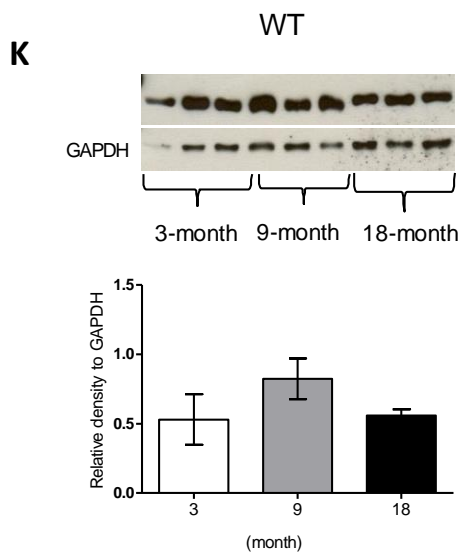
### Caveolin-2



### Flotillin-1



### Flotillin-2



1 **Supplementary Figure 1** Comparison of the expression of several proteins in  
2 the cortex of male WT and Tg mice aged 3, 9 and 18 months. Each section  
3 shows an immunoblot and densitometric analysis of the immunoblot.  
4 Comparison of APP expression in (A) WT mice and (B) Tg mice; comparison of  
5 Dynamin-1 expression in (C) WT mice and (D) Tg mice; comparison of Bin-1  
6 expression in (E) WT mice and (F) Tg mice; comparison of Caveolin-2  
7 expression in (G) WT mice and (H) Tg mice; comparison of Flotillin-1  
8 expression in (I) WT mice and (J) Tg mice; comparison of Flotillin-2 expression  
9 in (K) WT mice and (L) Tg mice. There were no significant differences between  
10 any groups for all proteins. n=3 mice for each age group.

11

Supplemental Digital content 2

Table showing the comparison of expression levels of endocytic proteins between WT and Tg London V717I mice

Protein/Age	WT mice <sup>1</sup>	Tg mice <sup>1</sup>
	OD ratio relative to GAPDH	
Clathrin, 3 month	0.7 ± 0.1	0.7 ± 0.1
Clathrin, 9 month	0.8 ± 0.1*	0.3 ± 0.1
Clathrin, 18 month	1.3 ± 0.2	1.9 ± 0.5
Dynamin 1, 3 month	1.1 ± 0.03	0.9 ± 0.1
Dynamin 1, 9 month	1.1 ± 0.02	0.9 ± 0.1
Dynamin 1, 18 month	1.1 ± 0.1	0.9 ± 0.1
PICALM bands 1&2, 3 month	0.8 ± 0.1	0.8 ± 0.1
PICALM band 3, 3 month	0.8 ± 0.1	0.7 ± 0.04
PICALM bands 1&2, 9 month	0.8 ± 0.1	0.9 ± 0.1
PICALM band 3, 9 month	0.8 ± 0.2	0.7 ± 0.1
Bin-1, 3 month	0.8 ± 0.1	0.7 ± 0.2
Bin-1, 9 month	2.3 ± 0.5	2.5 ± 0.5
Bin-1, 18 month	1.1 ± 0.2	0.9 ± 0.2
Caveolin-1, 3 month	1.1 ± 0.1	0.9 ± 0.3
Caveolin-1, 9 month	1.0 ± 0.2	0.9 ± 0.1
Caveolin-2, 3 month	0.9 ± 0.03	0.8 ± 0.1
Caveolin-2, 9 month	0.9 ± 0.04	0.9 ± 0.1
Caveolin-2, 18 month	1.0 ± 0.1	1.1 ± 0.1
Flotillin-1, 3 month	1.2 ± 0.1	1.2 ± 0.1
Flotillin-1, 9 month	1.6 ± 0.3	1.5 ± 0.1
Flotillin-1, 18 month	0.9 ± 0.1	0.8 ± 0.1
Flotillin-2, 3 month	1.0 ± 0.04	1.1 ± 0.2
Flotillin-2, 9 month	1.1 ± 0.1	0.9 ± 0.1
Flotillin-2, 18 month	0.9 ± 0.2	0.9 ± 0.1

<sup>1</sup> \* p,0.001, clathrin expression was significantly higher in 9 month WT mice compared to Tg mice analysed with an unpaired Student's t-test. There were no other significant differences between WT and Tg mice for any of the proteins at the different ages.

