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Hippocampal TMEM55B overexpression in the 5XFAD mouse model of Alzheimer's disease

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Abstract

Dysfunction of the endosomal-lysosomal network is a notable feature of Alzheimer's disease (AD) pathology. Dysfunctional endo-lysosomal vacuoles accumulate in dystrophic neurites surrounding amyloid β (Aβ) plaques and may be involved in the pathogenesis and progression of Aβ aggregates. Trafficking and thus maturation of these dysfunctional vacuoles is disrupted in the vicinity of Aβ plaques. Transmembrane protein 55B (TMEM55B), also known as phosphatidylinositol-4,5-bisphosphate 4-phosphatase 1 (PIP4P1) is an endo-lysosomal membrane protein that is necessary for appropriate trafficking of endo-lysosomes. The present study tested whether overexpression of TMEM55B in the hippocampus could prevent plaque-associated axonal accumulation of dysfunctional endo-lysosomes, reduce Aβ plaque load, and prevent hippocampal-dependent learning and memory deficits in the 5XFAD mouse models of Aβ plaque pathology. Immunohistochemical analyses revealed a modest but significant reduction in the accumulation of endo-lysosomes in dystrophic neurites surrounding Aβ plaques, but there was no change in hippocampal-dependent memory or plaque load. Overall, these data indicate a potential role for TMEM55B in reducing endo-lysosomal dysfunction during AD-like Aβ pathology.

Keywords

Alzheimer's disease; Lysosomes; Aβ; TMEM55B; PIP4P1

The endosomal-lysosomal network is comprised of dynamic intracellular vesicles that sort and traffic materials obtained from the extracellular environment as well as cellular proteins and organelles ready for autophagic recycling; appropriate function of this system is essential for cellular homeostasis (Nixon, 2017). The endosomal-lysosomal network is dysfunctional in Alzheimer's disease (AD), evident from the accumulation of autophagic vacuoles in the axons of dystrophic neurites due to disruption of retrograde trafficking,

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CONFLICT OF INTEREST

The authors have nothing to disclose.

acidification, and maturation of endo-lysosomes (Gowrishankar et al., 2015; Lie et al., 2022; Sadleir et al., 2016; Serrano-Pozo et al., 2016). Importantly, retrograde transport of these autophagic vacuoles is essential for their maturation to functional lysosomes (Nixon, 2017). When this lysosomal trafficking and maturation process is disrupted in AD pathology (Lie et al., 2022), it not only leads to cellular stress but also the accumulation of extracellular amyloid β (Aβ) (Lee et al., 2022). Additionally, accumulation of lysosomes around Aβ plaques is associated with increased oxidative stress (Wendt et al., 2022). Therefore, restoration of retrograde transport of endo-lysosomes may represent a therapeutic strategy to reduce not only dystrophic neurite pathology but also Aβ deposition and neurodegeneration during AD.

Transmembrane protein 55B (TMEM55B), also known as phosphatidylinositol-4,5-bisphosphate 4-phosphatase 1 (PIP4P1), is a key player in the retrograde trafficking of lysosomes in multiple cell types (Araki & Kontani, 2021; Rudnik et al., 2022; Takemasu et al., 2019; Willett et al., 2017). TMEM55B transcription is regulated by the master autophagic and lysosomal regulator TFEB (Willett et al., 2017) which is dysregulated in AD (Zhang & Zhao, 2015). Further, numerous genetic perturbation and pharmacological studies support the protective role of TFEB activation in a variety of neurodegenerative disease models (Gu et al., 2022; Song et al., 2021). The downstream mechanism of TFEB's protective actions in AD models are still poorly understood, but most likely involves a restoration of lysosomal homeostasis (Gu et al., 2022; Song et al., 2021). Knockout of TMEM55B leads to lysosomal stress and loss of vATPase function which is related to the acidification of lysosomes (Hashimoto et al., 2018). Others have found that activating TFEB ameliorates Aβ pathology (Xiao et al., 2015). The present study tests the hypothesis that overexpression of TMEM55B could ameliorate endo-lysosome dysfunction during Aβ pathology and mitigate Aβ plaque pathology by restoring function of endo-lysosomal trafficking and function. We and others have observed that lysosomal associated membrane protein 1 (LAMP1) is mislocalized to dystrophic neurites in 5XFAD models (Forner et al., 2021; Gowrishankar et al., 2015; Wickline et al., 2023) and human AD (Barrachina et al., 2006). In the present study, we find that hippocampal overexpression of TMEM55B modestly reduces peri-plaque LAMP1 immunopositivity. This suggest that TMEM55B activity may mitigate Aβ plaque-associated lysosomal dysregulation perhaps by promoting retrograde trafficking (Willett et al., 2017).

5XFAD mice (MMRRC #34848-JAX) and WT littermates of both sexes were treated with intrahippocampal stereotaxic injection (AP: −2.3, ML: ±1.5, DV: −2.3) of adeno associated virus serotype 9 (AAV9)-TMEM55B-GFP or phosphate buffered saline (PBS) as a control at 2 months of age to intervene in adult mice at the very earliest stages of Aβ plaque development(Forner et al., 2015; Oblak et al., 2021). GFP was fused to the N-terminal of human TMEM55B domain in the plasmid (a kind gift from Jose Martina and Rosa Puertollano) as previously described (Willett et al., 2017). AAV9 vectors were produced by Welgen, Inc. While the promoter was cytomegalovirus (CMV), AAV9 has robust tropism for neurons and displays high expression levels in the hippocampus (Aschauer et al., 2013). Our previous work demonstrates that about 400 transcripts, many related to ribosomal processing, are upregulated in AAV-GFP treated hippocampal neurons compared to PBS treated hippocampal neurons (Hopp et al., 2018), suggesting that the endo-lysosomal

pathway targeted herein is not greatly affected by AAV-GFP and that PBS can serve as an adequate control for the experiments herein. At 5 months of age and 90 days after injection, hippocampal-dependent spatial learning and memory was assessed using the Morris water maze (MWM) and non-hippocampal memory was assessed using Novel Object Recognition (NOR). However, NOR data was excluded from this publication because several mice failed to meet the minimum object interaction time of 20 seconds which resulted in some experimental groups being reduced such that a meaningful interpretation of the results was not possible. For MWM, Mice were trained for 5 days in two blocks of 3 60-second trials per block followed by a 60-second probe trial; mice that could not locate a visible platform within 60 seconds were removed from analysis. Following behavioral testing, mice were euthanized and brains processed for immunohistochemistry (1:300 rat anti-LAMP1, 1D4B from Developmental Studies Hybridoma Bank; 1:1000 rabbit anti-Aβ, D54D2; 1:1000 chicken anti-GFP, Aves;), imaged with brightfield fluorescence microscopy (Cytation 5, Figures 2A and 3) or confocal microscopy (Zeiss LSM 880, Figures 2B and C), and analyzed with ImageJ (see Supplemental methods).

Mice learned the location of the hidden platform during MWM regardless of treatment or genotype (repeated measures 3-way ANOVA, $F(4, 64) = 50.8$, $p<0.0001$, main effect of time, Figure 1A). Mice performed equivalently on the MWM probe trial of spatial memory regardless of genotype or treatment when measured as number of passes over the platform location (2-way ANOVA, $F(1, 16) = 0.05202$, $p = 0.8225$, main effect of genotype; $F(1, 16) = 0.7511$, $p = 0.3989$, main effect of treatment; Figure 1B) and distance to platform location (2-way ANOVA $F(1, 16) = 1.101$, $p = 0.3097$, main effect of genotype; $F(1, 16) = 2.936$, $p = 0.1059$; Figure 1D). Unexpectedly, 5XFAD mice spent significantly more time in the target quadrant than WT mice (2-way ANOVA, $F(1, 16) = 8.725$, $p = 0.0093$, main effect of genotype, Figure 1C). In mice injected with AAV-TMEM55B-GFP, there was widespread GFP expression in the hippocampus that extended to other brain areas that was not present in control mice (Figure 2A). Focal GFP expression was also found near plaques and in neurons and overlapped with LAMP1 endo-lysosomal staining (Figure 2B & C). Although LAMP1 signal intensity was equal among all experimental groups ($F(1, 14) = 0.7831$, $p = 0.3911$, main effect of genotype; $F(1,14) = 0.3892$, $p = 0.5428$, main effect of treatment; Figure 3B), WT mice displayed significantly greater LAMP1 positive area ($F(3, 14) = 27.96$, main effect of genotype; Figure 3C). Taken together, a change in area coverage without a change in intensity and the anatomical distribution within the representative images (Figure 3A and 2C middle panel) illustrate that 5XFAD Aβ pathology induces LAMP1 signal translocate away from cell bodies to instead accumulate in axonal swellings in peri-plaque regions (PPRs). Treatment with AAV-TMEM55B-GFP did not affect overall hippocampal LAMP1 ($F(1, 14) = 0.0002968$, $p = 0.9865$, main effect of treatment; Figure 3C) or Aβ (D54D2) plaque signal area ($p = 0.7542$; Figure 3D). However, to quantitatively measure changes in LAMP1 localization we developed a technique to measure LAMP1 percent area and intensity exclusively within the PPRs (Figure 3E, Supplemental Methods). Within PPRs, LAMP1 signal intensity was nearly two-fold greater than the average for the whole hippocampal section (2-way ANOVA, $F(1, 8) = 263.6$, $p < 0.0001$, main effect of ROI, Figure 3F). Further, 5XFAD mice treated with AAV-TMEM55B-GFP exhibit modestly but significantly reduced LAMP1 intensity than those treated with saline ($F(1, 8) = 8.363$, p

= 0.02, main effect of treatment, Figure 3F). Saline and AAV-TMEM55B-GFP injected mice did not differ in PPR LAMP1 positive area normalized as percent of hippocampal area (p = 0.9729).

Accumulation of Aβ into plaques is one of the two pathognomonic features of AD. However, abnormalities in endosomal-lysosomal processing is an early event that precedes the formation of Aβ plaques in numerous mouse models of Aβ pathology (Lee et al., 2022). Presenilin (PS) function, which is disrupted in patients with familial AD mutations in PS, is associated with dysfunctional lysosomal proteolysis and autophagy (Lee et al., 2010; Lie et al., 2022; Neely et al., 2011; Reddy et al., 2016); the 5XFAD mice used here express mutant PS1 with familial AD mutations. In the present study, enlarged LAMP1+ vacuoles surrounding Aβ plaques were observed, demonstrating the endosomal-lysosomal network dysfunction in 5XFAD mice (Figure 3). Defects in the endosomal-lysosomal network can impact numerous homeostatic cellular processes including cell signaling, molecular trafficking, and clearance pathways(Nixon, 2017). Dysfunction of the endosomal-lysosomal system is also directly linked with Aβ deposition (Lee et al., 2022), oxidative stress (Wendt et al., 2022), and synaptic dysfunction (Adalbert et al., 2009). However, these dystrophic neurites are not entirely disrupted (Adalbert et al., 2009), suggesting that restoration of trafficking could still result in neuroprotective benefit. Here, TMEM55B overexpression was tested as a potential mechanism to restore appropriate endo-lysosomal positioning, thus reducing LAMP1+ neuritic dystrophies and reducing accumulation of Aβ. Notably, we observed that LAMP1 intensity in regions surrounding Aβ plaques was reduced (Figure 3F), suggesting that TMEM55B expression reduced vacuolar accumulation in neurons surrounding Aβ plaques. However, when we assessed hippocampal LAMP1 and Aβ immunopositive percent area (Figure 3C–D), we did not observe a significant difference. Taken together, our results suggest that local TMEM55B overexpression had a subtle effect on Aβ plaque-associated LAMP1 pathology in 5XFAD mice. The 5XFAD mouse model which overexpression APP and presenilin with a combined 5 familial Alzheimer's disease mutations, is considered a pathologically "aggressive" model of Aβ pathology, meaning that Aβ-plaque pathology develops rapidly with respect to the lifespan of the mouse. Thus, perhaps our intervention would be more effective in a mouse model with a slower timescale for Aβ pathological progression. Despite the aggressive rate of pathological Aβ aggregation and lysosomal disruption in the 5XFAD and other FAD mice, they do not display robust age- or pathology-associated cognitive deficits on hippocampal and non-hippocampal memory tasks (Jankowsky & Zheng, 2017; Oblak et al., 2021), suggesting other pathological processes may be important for cognitive dysfunction in AD.

Few studies have examined the function of TMEM55B. TMEM55B catalyzes the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) into phosphatidylinositol 5-phosphate (PI5P) via its function as a 4-phosphatase. TMEM55B localizes to late endosomes and lysosomes and its overexpression mediates lysosomal degradation of internalized epidermal growth factor receptors (Ungewickell et al., 2005). TMEM55B also regulates surface expression of low-density lipoprotein receptor but not transferrin receptor or lipoprotein receptor 1 (Medina et al., 2014; Qin et al., 2020) suggesting its role in receptor trafficking may be cargo specific. TMEM55B regulates lysosome positioning and trafficking via interaction with the JIP4 motor protein adapter (Willett et al., 2017) independently from its

phosphatase function (Takemasu et al., 2019) but dependent on S-palmitoylation (Rudnik et al., 2022). Appropriate lysosomal trafficking is vital for maturation and acidification (Nixon, 2017). TMEM55B is also implicated in direct regulation of endo-lysosomal acidification, potentially independently of its trafficking functions (Hashimoto et al., 2018; Takemasu et al., 2019). Overall, these functions align with the data herein demonstrating that TMEM55B overexpression altered LAMP1 localization in 5XFAD mice. Although we were unable to detect any cognitive deficits in our 5XFAD mice, it is likely that the subtle changes observed in the intensity of LAMP1 surrounding Aβ plaques would be insufficient to modulate cognition in 5XFAD mice. Previous deep phenotyping of 5XFAD mice highlights the difficulty of modeling AD-like memory deficits in 5XFAD mice despite recapitulation of other AD-like phenotypes (Forner et al., 2015; Oblak et al., 2021).

Interestingly, while TMEM55B expression is dependent on TFEB (Willett et al., 2017), TMEM55B can actually inhibit TFEB nuclear translocation (Hashimoto et al., 2018) thus suppressing expression of lysosomal genes. Others have found that activating TFEB, the master transcriptional regulator of lysosomal biogenesis, ameliorates Aβ pathology (Xiao et al., 2015). TMEM55B-mediated feedback inhibition on TFEB translocation may underlie the subtle effect of TMEM55B overexpression on reducing peri-plaque LAMP1+ intensity in dystrophic neurites in 5XFAD mice documented herein. Notably, TMEM55B gene expression does not change throughout the lifespan of 5XFAD mice, but LAMP1, TFEB, and TFE3 gene expression increases after 12 months of age (Forner et al., 2021). These data suggest that early defects in endo-lysosomal function in 5-month-old 5XFAD mice herein may not be driven by transcriptional regulation but instead by post-translational processes and thus overexpression of TMEM55B may not be sufficient without altering post-translational processes that regulate TMEM55B function such as phosphorylation (Takemasu et al., 2019).

The study herein demonstrates that TMEM55B overexpression has subtle effects on the intensity of LAMP1 surrounding Aβ plaques in 5XFAD mice. This aligns with previous studies demonstrating the importance of TMEM55B in endo-lysosomal trafficking. However, additional studies using different Aβ pathology models, TMEM55B suppression, and more robust TMEM55B overexpression are necessary to characterize the overall role of TMEM55B in AD and in Aβ pathology. Further, it will be important to examine the interaction between TMEM55B and TFEB in the context of Aβ pathology to determine if TMEM55B's negative regulation of TFEB mitigates TFEB's therapeutic efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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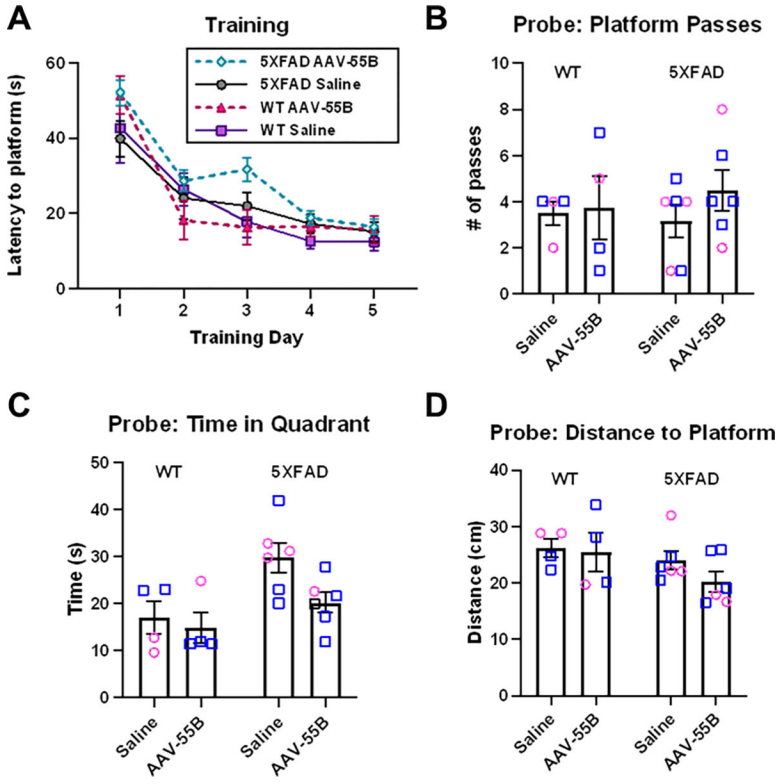

Figure 1. Mice display no spatial cognitive/memory deficits with respect to genotype or treatment.

(**A**) No genotype or treatment effects on learning during the training phase of the MWM in latency to locate the hidden platform. (**B**) No genotype or treatment effects on the probe trial of the MWM by number of passes over the platform location. (**C**) No genotype or AAV-TMEM55B-GFP (AAV-55B) effects on the probe trial of the MWM by time spent in the target quadrant. (**D**) No genotype or AAV-55B effects on the probe trial of the MWM by distance to platform location. N = 4–6 mice consisting of males (blue squares) and females (pink circles). Data are shown as mean with overlayed individual data points and error bars indicate standard error of the mean (SEM). (**A**) Repeated Measures Three-way ANOVA or (**B**) Two-way ANOVA was used to calculate statistical significance.

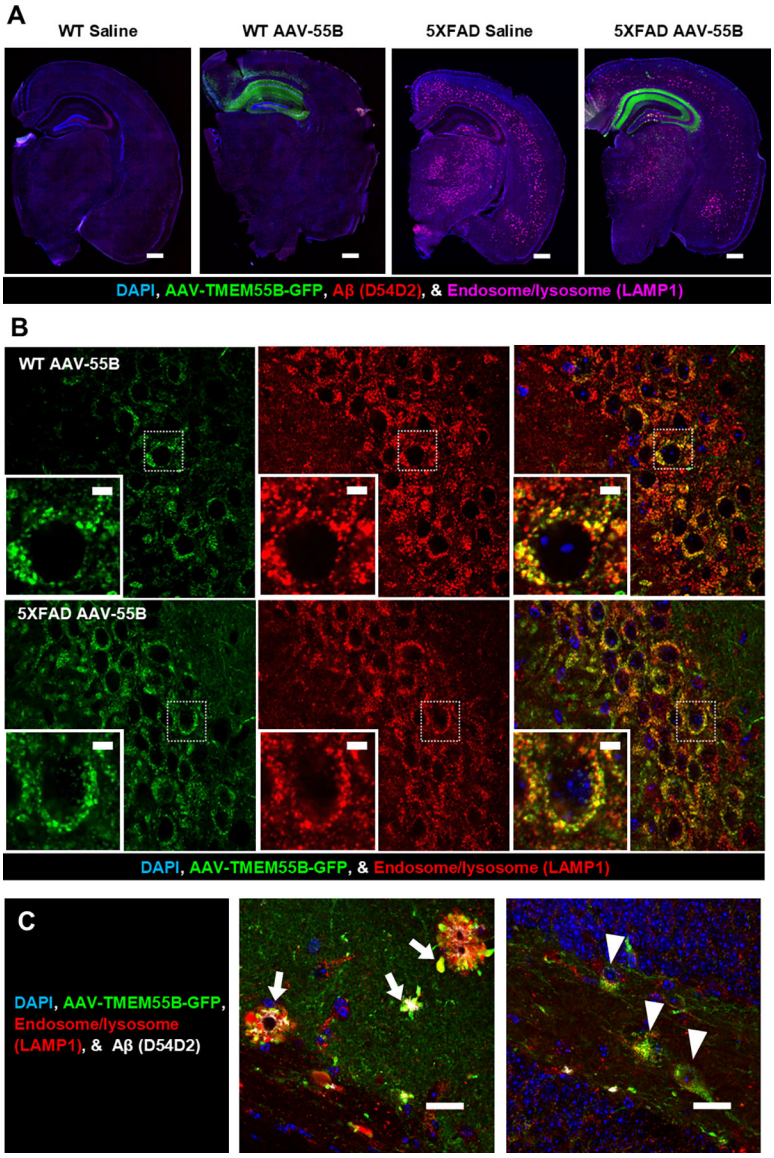

Figure 2. Single intrahippocampal AAV-TMEM55B-GFP injection efficiently labels hippocampus and TMEM55B-GFP colocalizes with endo/lysosomal marker LAMP1.

(**A**) Representative 20X brightfield montage images of coronal sections showing Aβ plaques, LAMP1, and GFP expression in the brain following injection with AAV-TMEM55B-GFP compared to saline-injected controls. Scale bars (bottom-right) are 500 μm wide. (**B**) Representative single z-plane 63X confocal images of Hippocampal CA3 neurons of AAV-TMEM55B-GFP injected WT (top) and 5XFAD (bottom) mice. Individual AAV-TMEM55B-GFP (left; green), LAMP1 (middle; red), and merged images with DAPI (right; blue) are shown. Overlapping GFP and LAMP1 is shown with yellow (green/red) pixels in merged image. Inset scale bars (top-right) are 50 μm wide. (**C**) Representative 63X single z-plane confocal of 5XFAD mouse CA1 plaques (white arrows) surrounded by AAV-TMEM55B-GFP/LAMP1 double positive dystrophic neurites (left) and hilar mossy neuron cell bodies double positive for AAV-TMEM55B-GFP/LAMP1.

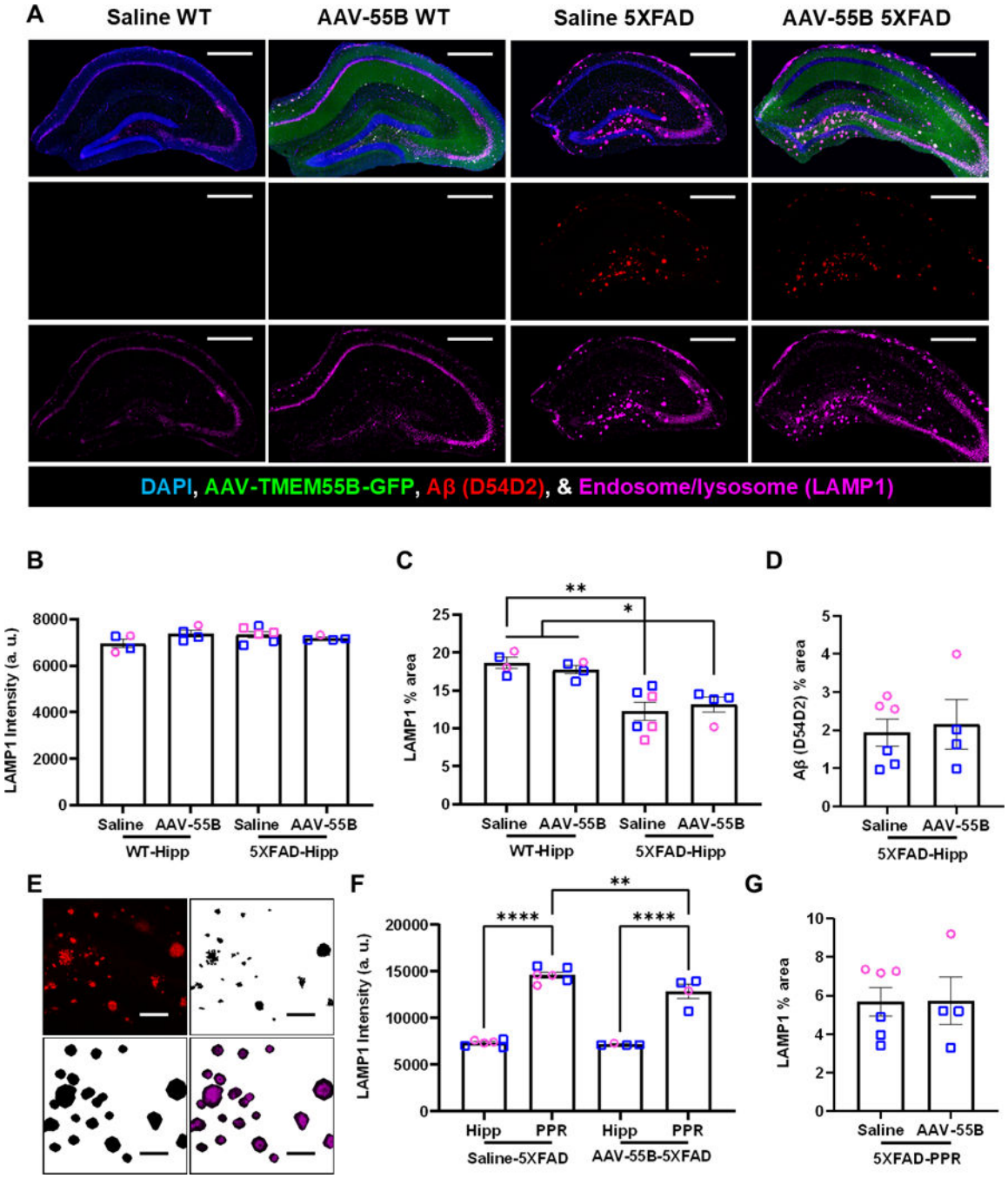

Figure 3. Local TMEM55B-GFP expression modulates accumulation of endo/lysosomes in dystrophic neurites surrounding plaques.

(**A**) Representative 20X images of hippocampus showing Aβ (D54D2) plaques, LAMP1, and GFP expression in the brain following injection with AAV-TMEM55B-GFP (AAV-55B) compared to saline-injected controls. Scale bars (top-right) are 500 μm wide. (**B**) No difference in hippocampal LAMP1 intensity with respect to genotype or AAV-55B (**C**) LAMP1 percent of hippocampal area is reduced in 5XFAD mice compared to WT (**D**) No difference in Aβ (D54D2) percent of hippocampal area between 5XFADs injected with

saline and with AAV-55B. (**E**) Demonstration of methods used to define peri-plaque regions (PPRs) including the following: raw, unprocessed Aβ (D54D2) image (top-left; red), Otsu threshold application and binarization of Aβ signal (top-right), automated object expansion to define PPR (bottom-left), visualization of PPR signal isolation within LAMP1 image (bottom-right; magenta). Scale bars (bottom-right) 100 μm wide. (**F**) LAMP1 fluorescence intensity is strongly enriched within the PPR, and PPR LAMP1 intensity is slightly reduced by AAV-55B treatment. (**G**) No difference in LAMP1 percent of hippocampal area within the PPR. N = 4–6 mice consisting of males (blue squares) and females (pink circles). Data are shown as mean with overlayed individual data points and error bars indicate SEM. Statistical significance was calculated using Two-way ANOVA with Tukey *post hoc* (**B** & **C**), Student's t test (**D** & **G**), and Repeated Measures Two-way ANOVA with Fisher's LSD *post hoc* (**F**). Significance of multiple comparisons *post hoc* analysis are denoted as *, **, and **** which correspond p values < 0.05, <0.01, and <0.0001, respectively.