ORIGINAL ARTICLE

Intracellular trafficking of TREM2 is regulated by presenilin 1

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Genetic mutations in triggering receptor expressed on myeloid cells 2 (TREM2) have been linked to a variety of neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral sclerosis, frontotemporal dementia and Parkinson's disease. In the brain, TREM2 is highly expressed on the cell surface of microglia, where it can transduce signals to regulate microglial functions such as phagocytosis. To date, mechanisms underlying intracellular trafficking of TREM2 remain elusive. Mutations in the presenilin 1 (PS1) catalytic subunit of the γ-secretase complex have been associated with increased generation of the amyloidogenic Aβ (amyloid-β) 42 peptide through cleavage of the Aβ precursor amyloid precursor protein. Here we found that TREM2 interacts with PS1 in a manner independent of γ-secretase activity. Mutations in TREM2 alter its subcellular localization and affects its interaction with PS1. Upregulation of PS1 reduces, whereas downregulation of PS1 increases, steady-state levels of cell surface TREM2. Furthermore, PS1 overexpression results in attenuated phagocytic uptake of Aβ by microglia, which is reversed by TREM2 overexpression. Our data indicate a novel role for PS1 in regulating TREM2 intracellular trafficking and pathophysiological function.

INTRODUCTION

Recently, several heterozygous missense mutations in triggering receptor expressed on myeloid cells 2 (TREM2) have been identified as risk factors for a number of neurodegenerative disorders including Alzheimer's disease (AD), amyotrophic lateral sclerosis, frontotemporal dementia and Parkinson's disease.[1–12] For example, the TREM2 R47H variant has been reported to confer an increased risk of AD in comparable odds ratios to APOE4,[8,9] the greatest known genetic risk factor for late-onset AD.[13]

TREM2 is a type I transmembrane protein comprising an extracellular, Ig-like V-type domain; a transmembrane domain; and an intracellular domain lacking any obvious signaling motifs (Figure 1f). Signal transduction induced by ligand engagement, for example, ApoE,[14–16] with the TREM2 extracellular domain is mediated through its association with DNAX-activating protein of 12 kDa (DAP12), which triggers intracellular signals through an immunoreceptor tyrosine-based activation motif.[17,18] TREM2 expression is primarily found in microglia, and mounting evidence indicates that TREM2 signaling is important in the regulation of microglial phagocytosis and inflammatory cytokine production.[19–30] Several disease-associated TREM2 mutations have been reported to reduce cell surface TREM2 distribution and impair microglial phagocytic function.[31,32] Therefore, the presence of TREM2 at the cell surface is important in mediating microglial functions such as phagocytosis. However, the precise mechanism by which TREM2 is distributed to the cell surface or intracellular compartments remains largely unknown.

Interestingly, it has been reported that TREM2 can be cleaved by the γ-secretase complex,[31,33] which is required for the generation of amyloid-β (Aβ) peptides that comprise pathological senile AD plaques.[34] Aberrant Aβ accumulation can trigger a cascade of neurodegenerative events including

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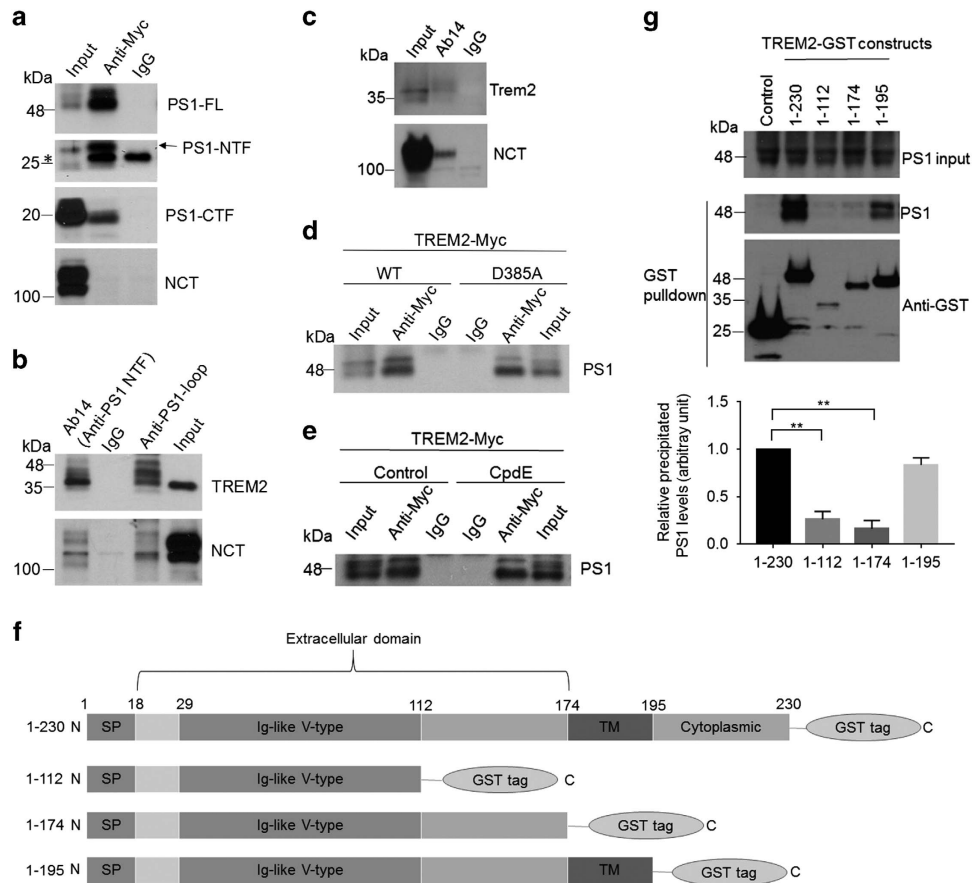Received 31 January 2017; revised 5 May 2017; accepted 31 May 2017

Figure 1 Presenilin 1 (PS1) interacts with TREM2. (**a**, **b**) PS1 constructs were transfected into HEK293 cells stably expressing TREM2 with a Myc-tag at the C terminus (HEK293-TREM2). (**a**) Cell lysates were immunoprecipitated with a Myc antibody or control IgG. Immunoprecipitated proteins were subjected to immunoblotting with an Ab14 antibody to detect full-length PS1 (PS1-FL) and the PS1 N-terminal fragment (NTF), an anti-PS1 loop antibody to detect the PS1 C-terminal fragment (CTF) and a nicastrin (NCT) antibody as indicated. (**b**) Cell lysates were immunoprecipitated with Ab14, anti-PS1 loop or control IgG and immunoblotted with Myc and NCT antibodies. (**c**) Lysates from BV2 microglial cells were immunoprecipitated with Ab14 and immunoblotted with NCT and mouse TREM2 antibodies. (**d**) Vectors expressing wild-type (WT) or mutant PS1 (D385A) were transfected into HEK293-TREM2 cells. Cell lysates were immunoprecipitated with Ab14 or control IgG, and PS1 was detected by immunoblotting. (**e**) Lysates from HEK293-TREM2 cells with or without Compound E (CpdE, a γ-secretase inhibitor) treatment were immunoprecipitated with Ab14 or control IgG, and PS1 was detected by immunoblotting. (**f**) Schematic representations of full-length (1–230) or truncated TREM2 constructs, all tagged with GST at the C terminus. SP, signal peptide; TM, transmembrane domain. (**g**) PS1 was co-expressed with full-length TREM2 or other TREM2 fragments as shown in **f** in HEK293 cells. Cell lysates were precipitated with Glutathione Sepharose beads and immunoblotted with the PS1 antibody Ab14 or an antibody against GST. PS1 co-precipitation levels were determined by densitometric analysis and normalized with respect to both PS1 expression and precipitated GST. **$P < 0.01$, $n = 3$, Student's t-test.

synaptic dysfunction, oxidative injury and eventual neuronal loss.[35,36] Importantly, microglia-mediated phagocytosis has been suggested to be involved in clearing extracellular Aβ deposits in the brain.[37] The γ-secretase complex is composed of four primary components: presenilins (PSs, including PS1 and PS2), anterior pharynx-defective-1 (APH-1), nicastrin (NCT) and presenilin enhancer-2 (PEN-2).[38,39] PS1 is the major presenilin component in the catalytically active γ-secretase complex, where autoproteolytically derived amino-terminal fragments (NTFs) and carboxyl-terminal fragments (CTFs) form functional heterodimers.[40] PEN-2 is known to cleave full-length PS1 to generate NTF and CTF constituents that form stable heterodimers. APH-1 is required for the assembly of premature components and for the proteolytic activity of the

complex, and NCT plays a primary role in the intracellular trafficking and the stability of the assembled complex.[41] Numerous PS1 mutations have been identified in early-onset familial AD patients.[42] Although PS1 is believed to be the catalytic subunit of γ-secretase, additional functions have been proposed for PS1, including the regulation of vesicular trafficking,[43,44] calcium homeostasis,[45,46] autophagy[47,48] and other cellular functions.[49]

Here we show that PS1 can interact with TREM2 to affect TREM2-mediated phagocytic capacity in microglia. Our results identify a novel mechanism underlying the regulation of intracellular TREM2 trafficking and provide insight into functional interactions between two fundamental AD risk components.

MATERIALS AND METHODS

Antibodies and reagents
Sulfo-NHS-LC-biotin, streptavidin agarose resin beads, Dynabeads Protein G, Turbofect Transfection Reagent and restriction enzymes were purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). Glutathione Sepharose 4B was purchased from GE Healthcare Life Sciences (Piscataway, PA, USA). The γ-secretase inhibitor Compound E was purchased from Millipore (San Diego, CA, USA). 6-carboxyfluorescein (FAM)-Aβ was purchased from AnaSpec (Fremont, CA, USA). The following antibodies were used in this study: mouse anti-Myc (for immunoblot, Life Technologies, Carlsbad, CA, USA); rabbit anti-Myc (for immunostaining) and rabbit anti-PDI (Cell Signaling Technology, Danvers, MA, USA); sheep anti-Myc (for immunostaining, Thermo Fisher, Carlsbad, CA, USA); sheep anti-TGN46 (GeneTex, Irvine, CA, USA); mouse anti-nicastrin (Abcam, Cambridge, MA, USA); rabbit anti-PS1-NTF (Ab14) antibody (developed in-house previously); mouse anti-PS1 loop (Millipore); goat anti-human TREM2 and sheep anti-mouse TREM2 (R&D Systems, Minneapolis, MN, USA); mouse anti-β-actin (Sigma, St Louis, MO, USA); rabbit anti-GST, normal mouse IgG and rabbit IgG (Santa Cruz Biotechnology, Dallas, TX, USA).

Cell culture and generation of stable cell lines
HEK293 cells and murine microglial BV2 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Corning, Corning, NY, USA) supplemented with 10% fetal bovine serum (HyClone, Piscataway, PA, USA). To generate HEK293 cells stably overexpressing human TREM2, we transfected cells with pcDNA3.1 vectors (Thermo Fisher) with Myc-tagged WT or mutated TREM2 cDNA inserts using Turbofect and selected for G418 resistance (400 μg ml^{-1}). A lentiviral vector comprising human PS1 cDNA-IRES-mCherry was purchased from GeneCopoeia, Rockville, MD, USA and packaged into lentiviral particles (Lv-PS1) through the viral core at Sanford Burnham Prebys Medical Discovery Institute. For stable PS1 overexpression, BV2 cells were transduced with Lv-PS1, and individual mCherry-positive cells were sorted by flow cytometry using a high-speed cell sorter (BD Biosciences, San Jose, CA, USA, FACSAria IIu), after which single clonal cell lines were expanded and characterized for expression.

RNA interference
Two PS1-targeting siRNAs (1, 5′-CCACACCATGTTGGAAATAAA-3′; 2, 5′-CCCACTTGTAAGTTTAAATAA-3′) and a scrambled control siRNA (Qiagen, Germantown, MD, USA) were transfected into BV2 cells using Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol.

Co-immunoprecipitation
HEK293 cells stably overexpressing WT or mutant TREM2 were transfected with or without pAG3 vectors for WT or D385A mutant PS1. Cells were lysed in lysis buffer (1% Nonidet P-40, 50 mM Tris-HCl, 150 mM sodium chloride, 2 mM EDTA, pH 7.4, supplemented with protease inhibitor mixture). Alternatively, BV2 microglia cells were lysed in CelLytic M buffer (Sigma) containing a protease inhibitor mixture. Lysates were incubated with normal IgG or the indicated antibodies together with Dynabeads Protein G at 4 °C overnight. Immunoprecipitated proteins were analyzed by immunoblotting.

GST pulldown
HEK293 cells were transfected with PS1 and various GST-tagged TREM2 constructs (Figure 1e). Cells were lysed and incubated with Glutathione Sepharose beads at 4 °C overnight. Precipitated proteins were analyzed by immunoblotting.

Cell surface biotinylation
Biotinylation was performed using a previously described protocol.[50] Cells were washed three times with ice-cold PBS/CM (phosphate-buffered saline containing 1.3 mM CaCl$_2$ and 1 mM MgCl$_2$) and incubated with 0.5 mg ml^{-1} sulfo-NHS-LC-biotin for 20 min at 4 °C. Cell lysates were precipitated with streptavidin beads overnight, and the precipitated biotinylated proteins were subjected to immunoblot analysis.

Immunoblot
Samples from co-IP, GST pulldown and cell surface biotinylation were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and probed using the indicated antibodies.

Immunostaining
HEK293 cells stably overexpressing WT or mutant TREM2 were transfected with PS1 expression constructs. Twenty-four hours after transfection, cells were fixed in 4% paraformaldehyde (PFA), permeabilized and blocked in 5% bovine serum albumin (BSA). The cells were then incubated with the indicated primary antibodies at 4 °C overnight. The cells were washed three times with PBS and then incubated with secondary antibodies conjugated to Alexa Fluor 488, 555 and 647 (Life Technologies). For cell surface labeling experiments, cells were blocked in 5% BSA after fixation and stained with a goat anti-human TREM2 antibody (epitope 19–174 amino acids, R&D Systems). The cells were then permeabilized and stained with a Myc antibody to detect total TREM2. Specimens were visualized using a confocal microscope (Zeiss, Oberkochen, Germany, LSM 710). Pearson's correlation coefficient was determined by Zen software to quantify colocalization between TREM2 and TGN46/PDI, between PS1 and TGN46/PDI, and between TREM2 and PS1. BV2 cells were fixed in 4% PFA and subjected to processing for imaging as described above.

Phagocytosis of FAM-Aβ
Treated BV2 microglial cells were seeded in 12-well plates at a density of 500 000 cells per well. After 24 h, the cells were incubated with media containing FAM-Aβ (500 nM) for 2 h. The cells were fixed with 4% PFA and visualized by confocal microscopy (Zeiss LSM 710). Alternatively, extracellular and cell surface FAM-Aβ was quenched by incubation with 0.4% trypan blue in PBS (pH 4.4) for 1 min. The cells were then trypsinized, washed with ice-cold PBS and subjected to flow cytometry analysis using an LSRFortessa X-20 cell analyzer (BD Biosciences).

Statistical analyses
Statistical analyses were performed with GraphPad Prism (La Jolla, CA, USA). N represents the number of biological replications. Student's t-test or one-way ANOVA was used. All data are presented as the mean ± s.d.

RESULTS
To identify proteins that may regulate TREM2 trafficking, we generated a human embryonic kidney 293 cell line stably

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overexpressing Myc-tagged TREM2 (HEK293-TREM2) and performed co-immunoprecipitation (co-IP) assays to screen for TREM2-interacting components. Using this system, we identified positive interactions between TREM2 and PS1: with PS1 overexpression in HEK293-TREM2 cells, a Myc antibody immunoprecipitated full-length PS1, PS1-NTF and PS1-CTF, while no interaction between TREM2 and NCT was detected (Figure 1a). Conversely, TREM2 was co-precipitated using antibodies against PS1-NTF (Ab14) or PS1-CTF (anti-PS1 loop) (Figure 1b). Moreover, we found that endogenous TREM2 co-immunoprecipitated with endogenous PS1 in BV2 microglial cells (Figure 1c). The catalytically inactive dominant negative PS1 mutant (D385A)[51] retained interaction with TREM2 comparable to wild-type (WT) PS1 (Figure 1d). Additionally, inhibition of γ-secretase activity with Compound E (CpdE) did not affect PS1/TREM2 co-precipitation (Figure 1e). These results demonstrate that PS1 interacts with TREM2 and that the interaction occurs irrespective of γ-secretase activity.

To map the domain(s) required for TREM2/PS1 interaction, we co-transfected PS1 and glutathione S-transferase (GST)-tagged full-length or truncated TREM2 into HEK293 cells and assayed for interactions between PS1 and TREM2-GST constructs in Glutathione Sepharose precipitates (Figures 1f and g). We found that TREM2 constructs comprising the transmembrane region (175–195 amino acids) co-precipitated with PS1, whereas TREM2 fragments lacking the transmembrane region pulled down much less PS1 (Figure 1g), suggesting that the TREM2 transmembrane domain is crucial for TREM2/PS1 interaction.

Given that several disease-associated mutations in TREM2 have been previously described to alter its intracellular localization,[31,32] we generated HEK293 cell lines stably over-expressing TREM2 R47H, T66M and T96K mutants. We found that WT, R47H and T96K TREM2 predominantly localized in the Golgi apparatus, as seen by high colocalization coefficients observed with the Golgi marker TGN46, (Figures 2a and c), whereas T66M TREM2 primarily localized in the endoplasmic reticulum (ER) as indicated by colocalization with the ER marker PDI (Figures 2b and c). In addition, we observed that T66M mutation markedly reduced cell surface TREM2 expression, whereas R47H and T96K mutations did not affect surface expression of TREM2 (Supplementary Figure 1). PS1 could also be found in TGN46- or PDI-labeled structures (Figures 2a–c). Partial TREM2/PS1 colocalization was observed with the various TREM2 variants: specifically, staining overlap (white color) was observed among WT, R47H or T96K TREM2, PS1 and TGN46, and overlapping staining of T66M TREM2, PS1 and PDI was also apparent (Figures 2a–c). Although mutations in TREM2 did not influence PS1/TREM2 colocalization (Figure 2c), comparatively weaker T66M TREM2 interaction with PS1 was observed relative to the interaction between WT TREM2 and PS1, based on co-IP assays using the PS1 antibody Ab14 (Figure 2d). Moreover, we also observed colocalization between endogenous TREM2 and PS1 in the

BV2 microglial cell line (Pearson's colocalization coefficient, 0.41 ± 0.11), primarily in Golgi-like compartments (Figure 3).

PS1 has been reported to regulate cell surface delivery of several transmembrane proteins such as amyloid precursor protein.[43,44] We next determined whether PS1 can affect surface expression of TREM2 by cell surface biotinylation assays. PS1 overexpression in HEK293-TREM2 cells resulted in a significant reduction in cell surface TREM2 levels with no change in total TREM2 levels (Figure 4a). Furthermore, we found that PS1 overexpression reduced cell surface TREM2 levels in the presence of the γ-secretase inhibitor CpdE, indicating that PS1-dependent depletion of TREM2 at the cell surface occurs independently of γ-secretase activity (Figure 4a). To confirm these effects in a microglia-derived cell line, we generated a murine microglial BV2 cell line stably overexpressing PS1, transduced with a lentiviral delivery system (BV2-PS1). We observed a reduction in endogenous TREM2 at the cell surface in BV2-PS1 cells compared with control BV2 cells (Figure 4b). To further clarify the regulatory effects of PS1 on TREM2 trafficking, we examined the effect of PS1 down-regulation on cell surface TREM2 expression in BV2 cells. We found that PS1 knockdown significantly increased cell surface TREM2 levels (Figure 4c). As expected, cell surface NCT levels were concomitantly reduced upon PS1 knockdown (Figure 4c). We then investigated whether upregulation of other γ-secretase components such as NCT may have similar effects, and we found that overexpression of NCT had little effect on cell surface TREM2 distribution (Figure 4d). Together, these results indicate that TREM2 cell surface distribution is regulated by PS1 in a manner independent of the γ-secretase complex.

The presence of TREM2 at the cell surface is important for transducing extracellular ligand signals to enact a microglial response. Several studies have reported that reduction of cell surface TREM2 expression in microglia cells can attenuate phagocytic function.[23,31] In support of this notion, transient overexpression of PS1 reduced phagocytic uptake of FAM-labeled Aβ42 within a 2-h incubation period in BV2 cells (Figure 5a). We further confirmed a reduction in FAM-Aβ42 uptake in BV2-PS1 cells by flow cytometry analysis (Figure 5b). To determine whether this reduction was TREM2-dependent, we transduced BV2-PS1 cells with lentiviruses expressing human TREM2. Strikingly, BV2-PS1 cells overexpressing TREM2 (BV2-PS1+TREM2) exhibited comparable phagocytic activity to control BV2 cells lacking PS1 and TREM2 over-expression (Figure 5b). In addition, we found that cell surface expression of TREM2 in BV2-PS1+TREM2 cells is comparable to that in BV2-control cells (Supplementary Figure 2). We observed no significant difference between the control and PS1 knockdown samples in bulk microglial phagocytic uptake (Figure 5c). Together, these results indicate that PS1 and TREM2 interact to reduce cell surface TREM2 distribution and downstream phagocytotic function.

DISCUSSION
TREM2 has been previously reported to be a substrate of the γ-secretase complex,[31,33] which suggests that TREM2 proteolysis
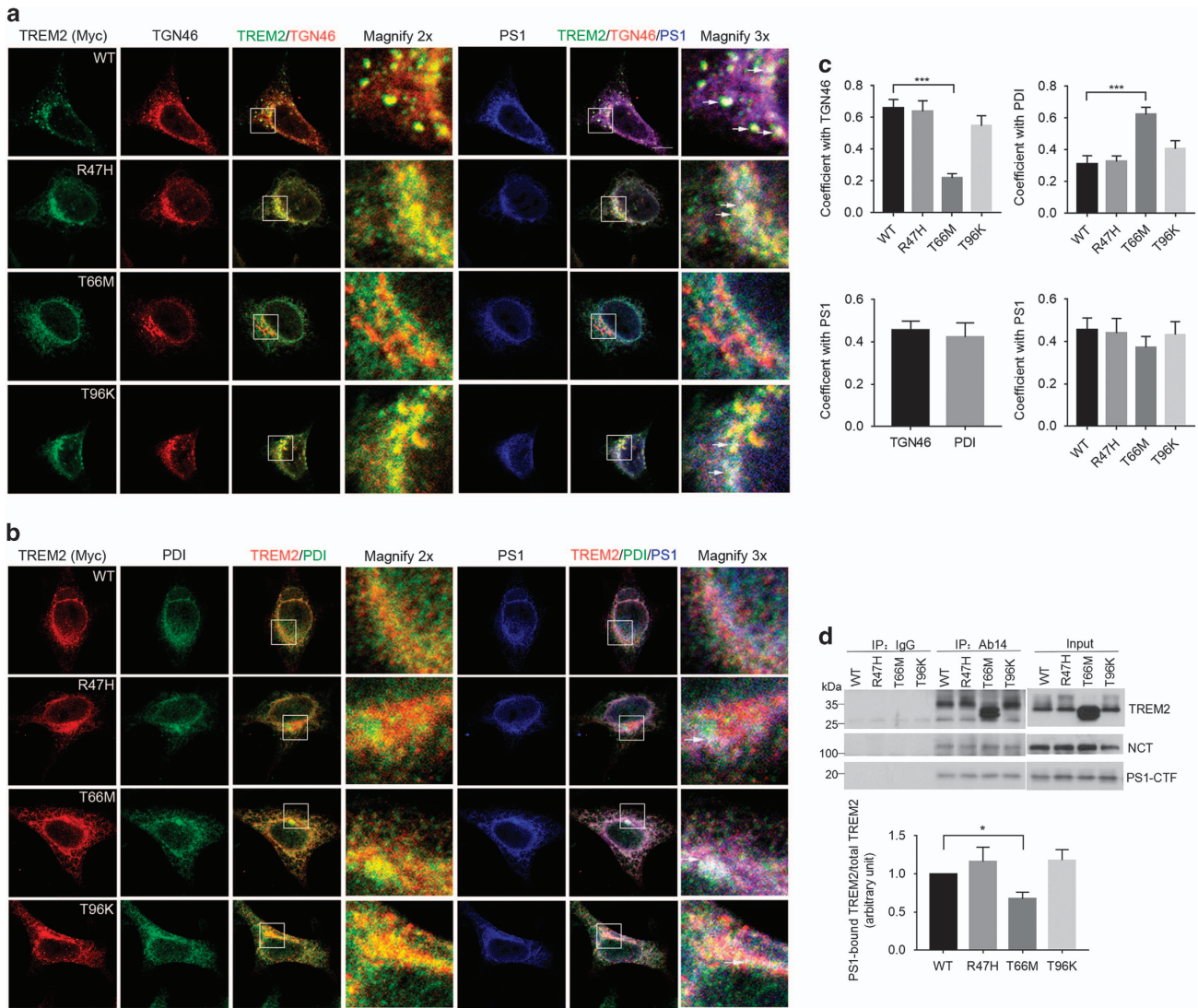
Figure 2 Mutations in TREM2 affect colocalization and interactions between TREM2 and presenilin 1 (PS1). PS1 was transfected into HEK293 cells stably expressing Myc-tagged TREM2 WT or TREM2 mutants as indicated. (**a**, **b**) Cells were then subjected to immunostaining with antibodies against Myc, PS1, and TGN46 (a marker for the Golgi, **a**) or PDI (a marker for the ER, **b**). White arrows in magnified images indicate colocalizing overlap for TREM2, PS1 and TGN46/PDI. Scale bars for **a**, **b**, 10 μm. (**c**) Quantification of colocalized signals. Pearson's correlation coefficient is shown. ***$P<0.001$, $n=3$ independent experiments, one-way ANOVA with Dunnett's *post hoc* analysis. (**d**) Cell lysates were immunoprecipitated with the PS1 antibody Ab14 or normal IgG. TREM2, NCT and PS1-CTF were detected by immunoblotting. The levels of precipitated TREM2 WT and mutants were normalized to the input. *$P<0.05$, $n=3$, Student's *t*-test.
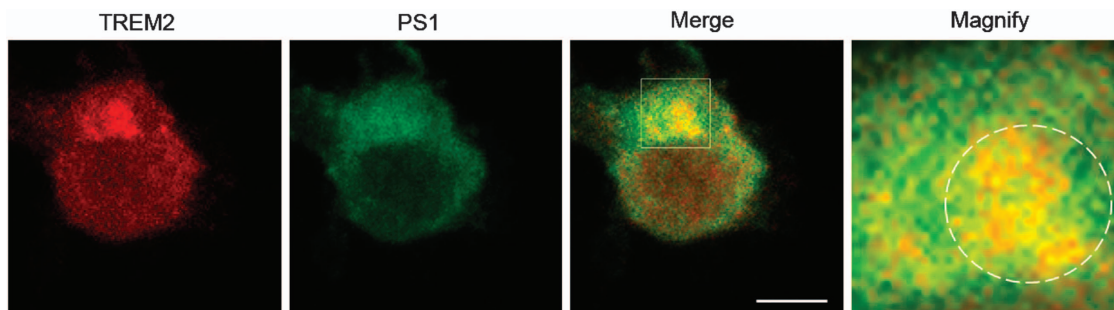

Figure 3 Endogenous TREM2 partially colocalizes with endogenous presenilin 1 (PS1) in microglial BV2 cells. BV2 cells were immunostained with antibodies against PS1 and mouse TREM2. White circles in magnified images indicate some colocalizing overlap between TREM2 and PS1 in Golgi-like structures. Scale bar, 5 μm.
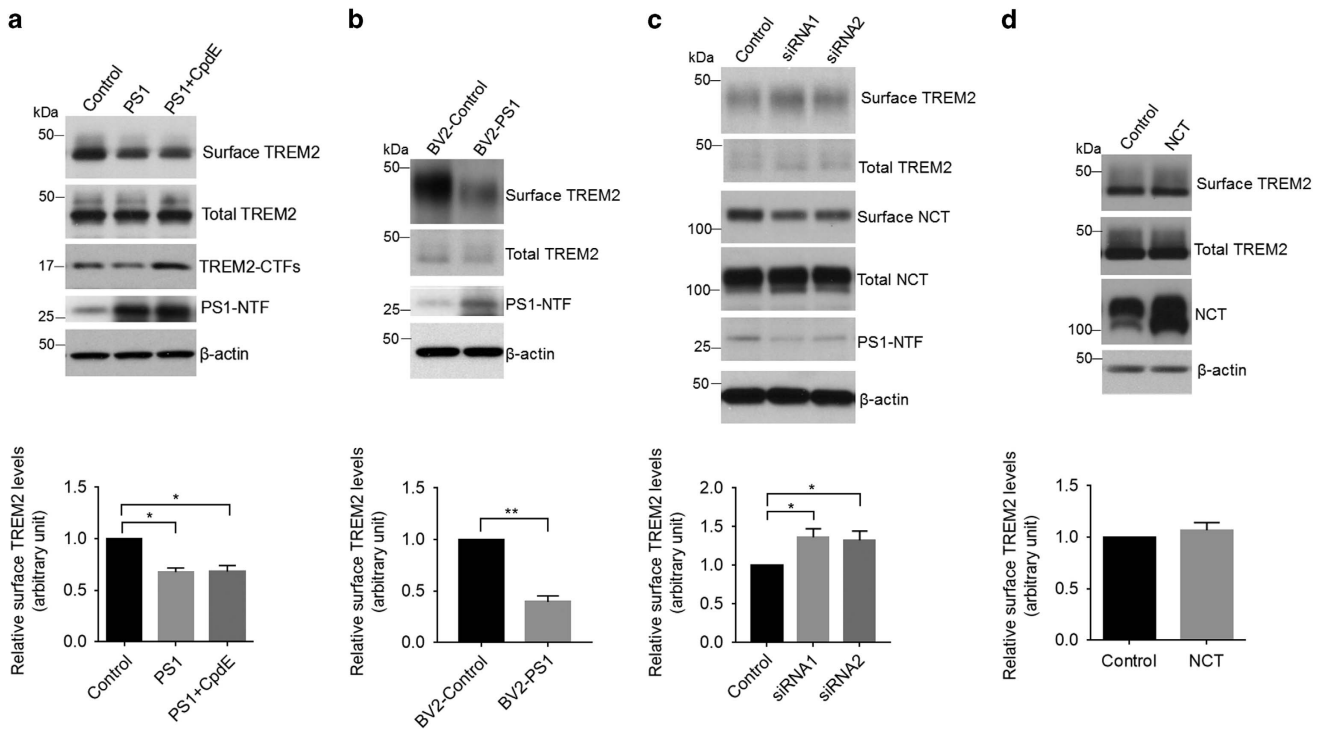
Figure 4 Upregulation of presenilin 1 (PS1) reduces steady-state levels of TREM2 at the cell surface. (**a**) Following PS1 overexpression, HEK293-TREM2 cells were treated with or without the γ-secretase inhibitor Compound E (CpdE) and subjected to cell surface biotinylation assay. Precipitates from streptavidin-agarose beads were immunoblotted for biotinylated TREM2 and total TREM2 levels (levels of TREM2 in 2% total cell lysates). $*P < 0.05$, $n = 3$, one-way ANOVA with Sidak *post hoc* analysis. (**b**) The level of endogenous TREM2 at the surface of microglial BV2 cells stably expressing PS1 (BV2-PS1) was determined by biotinylation. $**P < 0.01$, $n = 3$, Student's *t*-test. (**c**) BV2 cells were transfected with a scrambled control siRNA or PS1-targeting siRNAs for 72 h. The level of cell surface TREM2 was determined by surface biotinylation. $*P < 0.05$, $n = 3$, Student's *t*-test. (**d**) Cell surface expression of TREM2 in HEK293-TREM2 cells with NCT overexpression, as determined by biotinylation. $n = 3$, Student's *t*-test.

could influence cell surface TREM2 distribution and function. Coordination of γ-secretase subunits including PSs, APH-1, NCT and PEN-2 is a prerequisite for γ-secretase-mediated proteolysis.[41] Since total full-length TREM2 levels were unaffected by PS1 overexpression, this suggests that PS1 upregulation alone cannot enhance TREM2 proteolysis. Although inhibition of γ-secretase activity has recently been shown to reduce TREM2-mediated phagocytosis, which may be due to competitive binding between accumulated TREM2-CTF and DAP12, thereby impairing interactions between full-length TREM2 and DAP12 that are required for phagocytosis,[52] our results demonstrate that PS1-dependent TREM2 trafficking remains unaffected by γ-secretase inhibition. Therefore, the effects of PS1 overexpression on TREM2 trafficking and microglial phagocytosis are likely not due to γ-secretase-dependent TREM2 cleavage in our system. Moreover, PS1 knockdown also had no effect on microglial phagocytosis. We speculate that cell surface full-length TREM2 and TREM2-CTF are both increased with PS1 knockdown, whereby both full-length TREM2 and TREM2-CTF can compete for DAP12 binding, thus producing no net effect on DAP12 signaling. On the whole, it is likely that PS1 can regulate the phagocytic function of TREM2 through both γ-secretase-dependent proteolytic and γ-secretase-independent trafficking roles.

Since TREM2 can be cleaved by (ADAM10A disintegrin and metalloproteinase domain-containing protein 10) on the cell surface,[31] we speculate that overexpression of PS1 may reduce TREM2 ectodomain shedding. Although we do not know at this time how PS1 regulates TREM2 intracellular trafficking, several possibilities exist: one possibility is that PS1 affects the TREM2 trafficking from the Golgi/TGN; alternatively, PS1 may affect TREM2 internalization from the cell surface; a third possibility is that PS1 modulates the recycling of TREM2 from the endosomes and TGN to the cell surface. Future study may yield further information regarding how TREM2/PS1 interplay can affect consequent TREM2 distribution.

Various mutations identified in the TREM2 ectodomain exert differing effects on TREM2 structure. While R47H and T96K do not drastically affect TREM2 folding, T66M results in misfolding of TREM2 protein.[53] Indeed, this may explain the altered subcellular localization of T66M TREM2 and reduced T66M/PS1 interaction that we observed. In addition to its role in mediating proteolytic catalysis within the γ-secretase complex, PS1 has been shown to execute other functions including the regulation of calcium homeostasis,[45,46] autophagy[47,48] and trafficking of various membrane proteins such as amyloid precursor protein.[43,44] Our results here point to a novel role for
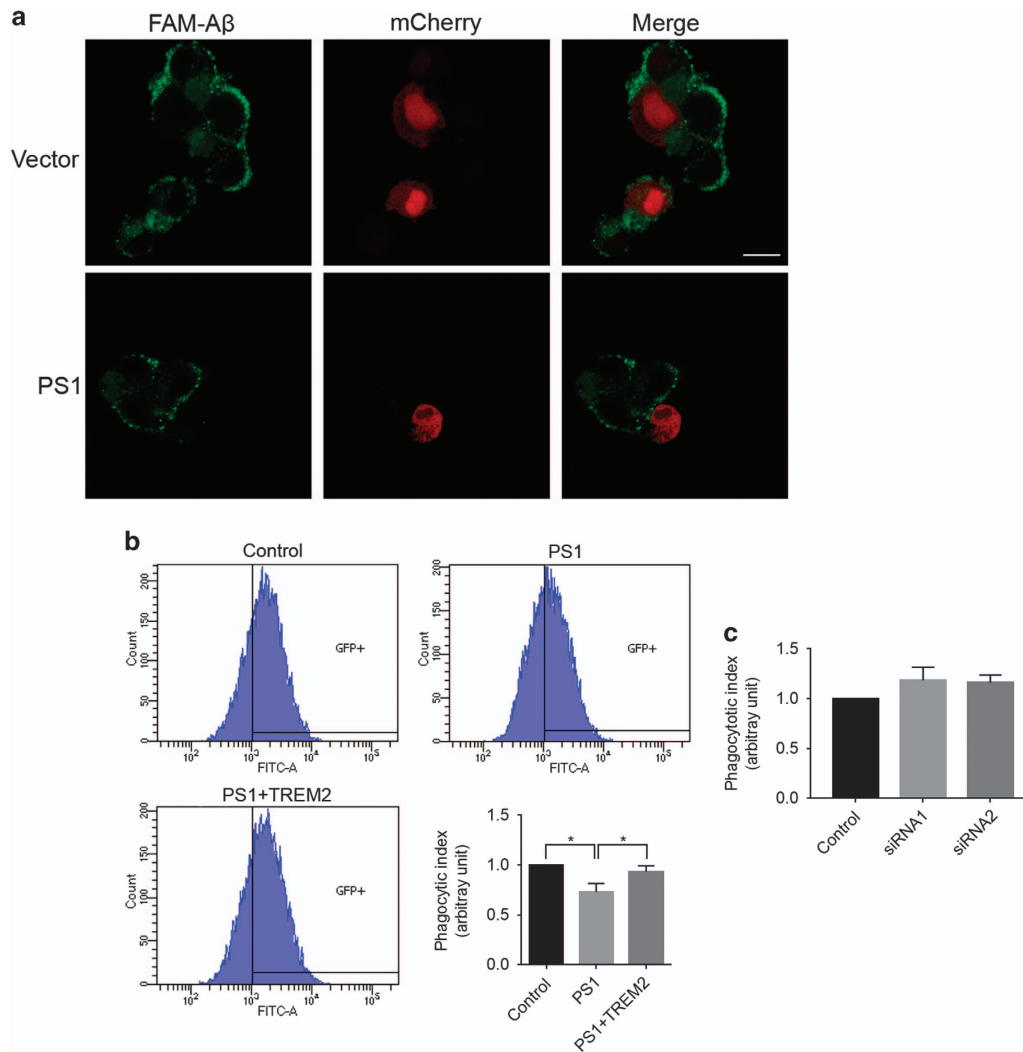
Figure 5 Overexpression of presenilin 1 (PS1) impairs TREM2-mediated phagocytosis in microglial cells. (**a**) PS1 and mCherry were co-transfected into BV2 microglial cells. The cells were then incubated with 6-carboxyfluorescein (FAM)-labeled Aβ42 for 2 h. FAM-Aβ42 uptake was analyzed by fluorescence microscopy. Scale bars, 10 μm. (**b**) Phagocytosis of FAM-Aβ42 in BV2 cells stably expressing PS1 as determined by flow cytometry. *$P < 0.05$, $n = 3$, one-way ANOVA with Sidak *post hoc* analysis. (**c**) Phagocytosis of FAM-Aβ42 in BV2 cells following PS1 knockdown as determined by flow cytometry. $n = 3$, Student's *t*-test.

PS1 in regulating microglial phagocytosis through the regulation of TREM2 trafficking. Given that TREM2 signaling is also involved in the production of inflammatory cytokines,[25,26,28] it may be of interest to further determine whether PS1 has a γ-secretase-independent role in mediating neuroinflammation. As neuroinflammatory processes such as microglial activation and cytokine generation are commonplace in multiple neurodegenerative diseases such as AD,[22,54] modulation of PS1/TREM2 trafficking may present alternative targeting strategies to treat AD. Since the use of γ-secretase inhibitors to inhibit Aβ generation has largely failed clinically,[55] alternative strategies such as targeting PS1/TREM2 trafficking may be more effective in reversing cognitive decline near the onset of AD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Author contributions: YZ, QW and HX conceived the study. YZ and XL designed and performed most of the experiments. TH carried out the GST pulldown assays. LLJ performed some of the phagocytosis assays. MZ made some of the constructs used in this study. TH, ZT, XW,

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YWZ, IHC and GB provided additional advice on the experimental design and execution. YWZ supervised MZ. YZ, XL and HX wrote the manuscript. TH and YWZ edited the manuscript.

PUBLISHER'S NOTE

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Supplementary Information accompanies the paper on Experimental & Molecular Medicine website (http://www.nature.com/emm)