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Novel TREM2 splicing isoform that lacks the V-set immunoglobulin domain is abundant in the human brain

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Abstract

TREM2 is an immunoglobulin-like receptor expressed by certain myeloid cells, such as macrophages, dendritic cells, osteoclasts and microglia. In the brain, TREM2 plays an important role in the immune function of microglia, and its dysfunction is linked to various neurodegenerative conditions in humans. Ablation of TREM2 or its adaptor protein TYROBP causes Polycystic Lipo-Membranous Osteodysplasia with Sclerosing Leukoencephalopathy (also known as Nasu-Hakola disorder) with early onset of dementia, while some missense variants in TREM2 are associated with an increased risk of late-onset Alzheimer's disease. The human *TREM2* gene is subject to alternative splicing, and its major, full-length canonical transcript encompasses 5 exons. Herein, we report a novel alternatively spliced *TREM2* isoform without exon 2 (Δe2), which constitutes a sizable fraction of *TREM2* transcripts and has highly variable inter-individual expression in the human brain (average frequency 10%; range 3.7–35%). The protein encoded by Δe2 lacks a V-set immunoglobulin domain from its extracellular part but retains its transmembrane and cytoplasmic domains. We demonstrated Δe2 protein expression in TREM2-positive THP-1 cells, in which the expression of full-length transcript was precluded by CRISPR/Cas9 disruption of the exon 2 coding frame. Similar to the full-length TREM2, Δe2 is sorted to the plasma membrane and is subject to receptor shedding. In "add-back" experiments, Δe2 TREM2 had diminished capacity to restore phagocytosis of amyloid beta peptide and promote interferon type I response as compared to full-length TREM2. Our findings suggest that changes

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AUTHORSHIP

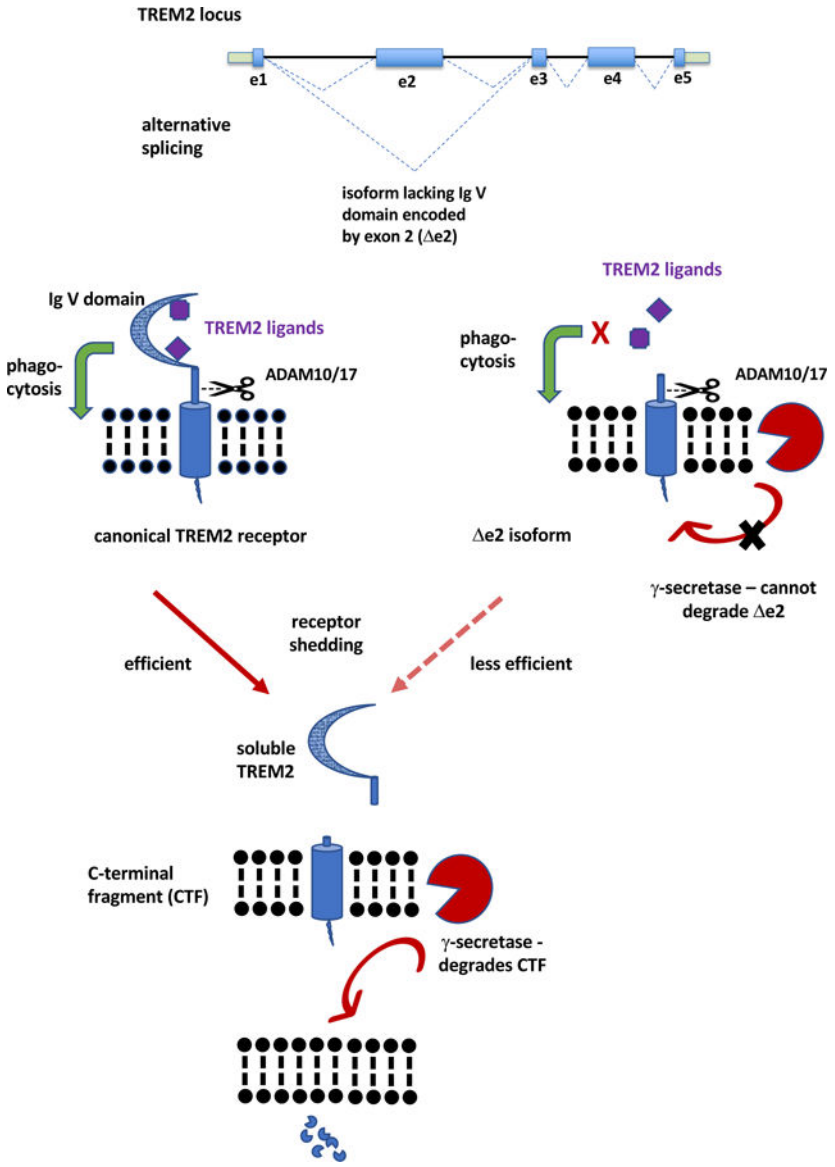
K. K. designed and performed the experiments, analyzed the results, and wrote the manuscript, I. K., M.M., N. B., N.L. and W-M. C. performed the experiments and analyzed the results; W. H. R. and O.K. supervised the studies, analyzed and interpreted the results, wrote the manuscript and acquired funding.

DISCLOSURE

The authors declare no conflicts of interest.

in the balance of two mutually exclusive TREM2 isoforms may modify the dosage of full-length transcript potentially weakening some TREM2 receptor functions in the human brain.

Graphical Abstract



1. Introduction

Triggering **r**eceptor **e**xpressed on **m**yeloid cells **2** (TREM2) is an immunoglobulin-like molecule expressed by some myeloid cells, such as macrophages, dendritic cells, osteoclasts and microglia. It is an important regulator of the innate immune response and its deficiency is linked to several neurodegenerative disorders in humans (1). TREM2 forms a signaling receptor complex with TYROBP adaptor protein. Loss-of-function bi-allelic mutations in TREM2 or TYROBP cause Polycystic Lipo-Membranous Osteodysplasia with Sclerosing

Leukoencephalopathy (PLOSL; OMIM # 618193 and # 221770), a recessive disorder that presents with early onset dementia and bone cysts (2), or familial Frontotemporal Dementia without bone cysts (FTD) (3),(4),(5). PLOSL patients appear cognitively and neurologically normal till the 4th decade, highlighting a neuroprotective role of TREM2 in adult/aging microglia. The TREM2 missense variants R47H and R62H are associated with an increased risk of Alzheimer's disease (AD) (OR 2.8–4.6 (6),(7) and 1.4–2.4 (8),(9), respectively), and R47H may also be linked to Parkinson's disorder (10) and Amyotrophic Lateral Sclerosis (11).

TREM2 is involved in multiple aspects of myeloid cell function (12). In microglia, it regulates chemotaxis, enhances phagocytosis (13) and influences survival, proliferation and differentiation (14). Recently, we demonstrated a novel role of TREM2 in the regulation of antiviral interferon type I response (IFN I) in myeloid cells and exaggerated response in the brain of R47H carriers with AD (15). TREM2 binds lipids and lipoproteins (16), including the known AD risk factors ApoE and ApoJ/CLU (17). Another TREM2 ligand is amyloid beta peptide (Aβ) (18), a major constituent of amyloid deposits in AD brains. TREM2 binding to Aβ activates microglia cytokine production and degradation of internalized peptide. A soluble form of TREM2, a product of cleavage or alternative splicing, may have a separate function as an extracellular signaling molecule that promotes cell survival (19).

The human *TREM2* gene is represented by several alternatively spliced transcripts (Fig. 1A). A major canonical form, ENST00000373113, comprises 5 exons and encodes the full-length transmembrane receptor protein. Two minor isoforms are products of alternative splicing of exon 4 that encodes the transmembrane domain. In ENST00000338469, exon 4 is skipped, while in ENST00000373122 exon 4 has an alternative start that changes its coding sequence. As a result, both isoforms lack the transmembrane domain and are thought to be secreted proteins. All three *TREM2* isoforms are expressed in the human cortex (8). Herein, we report a novel alternatively spliced *TREM2* transcript lacking exon 2 (Δe2) which constitutes a sizable fraction of *TREM2* transcripts in the brain and has functional activities different from the canonical full-length TREM2.

2. Materials and Methods

2.1. Brain tissues for RNA analyses.

The study was approved by the Institutional Review Board of the University of Washington. Postmortem human brain tissues of cognitively normal individuals (N=14) were provided by the UW Neuropathology Core Brain Bank (Table 1S). Permission was obtained from donors for brain autopsy and the use of tissues for research purposes. The average post-mortem interval was 4.2 h (range 2:30–6:25 h). Tissue samples were flash-frozen at the time of autopsy and stored at −80°C.

2.2. Cell lines and derivatives.

Human myeloid cell lines THP-1 and MOLM-13 were obtained from the ATCC. Cells were cultured in RPMI supplemented with GlutaMax and 10% fetal bovine serum. TREM2 knockouts THP-1 (KO) were engineered using CRISPR/Cas9 disruption of the coding

frame in exon 2 (15). Coding sequences of human full-length *TREM2* (ENST00000373113) and the Δe2 isoform were synthesized and cloned into the doxycycline-inducible lentiviral pCW57-MCS1–2A-MCS2 vector (Addgene, #71782). For "add-back" experiments, TREM2 KO cells were transduced with the corresponding full-length or Δe2 lentiviral expression constructs. Efficiencies of transduction were 90% and 75% for the FL and Δe2 constructs, respectively. After puromycin selection, resistant cells were harvested and cryopreserved; low passage cultures were used for all experimentation. Ectopic TREM2 expression was induced with doxycycline (100 ng/ml) and validated by qRT-PCR and by Western blot.

2.3. Chemicals and inhibitors.

Phorbol 12-myristate 13-acetate (PMA, Millipore-Sigma, CAS 16561–29-8), doxycycline (Thermo Fisher, CAS 10592–13-9), puromycin (Gibco, #A1113803), recombinant human IL-4 (PeproTech, #200–04); gamma-secretase inhibitor IX (DAPT, Millipore-Sigma, CAS 208255–80-5).

2.4. RNA isolation and cDNA synthesis.

Total RNA from cultured cells was isolated with the RNeasy kit (Qiagen; #74106). Total RNA from cryopreserved brain autopsies was isolated using TRIZOL reagent (Invitrogen). cDNA was synthesized using SuperScript III First-Strand Synthesis kit with oligo(dT) primers (Invitrogen, #18080051).

2.5. TREM2 cDNA cloning.

Total RNA was isolated from frontal cortex of four subjects and converted to cDNA. *TREM2* sequence was amplified using primers positioned within untranslated 5′ and 3′ regions; forward primer: 5′-gcagttcaagggaaagacga-3′; reverse primer: 5′-tccagctaaatatgacagtcttgg-3′. PCR products were gel-purified, cloned into pCR-XL-Topo vector (Invitrogen, # K4700) and sequenced.

2.6. qRT-PCR assays

were performed on a 7500 Real-Time PCR System (Applied Biosystems) in technical duplicates or triplicates. *TBP* (TATA-Box Binding Protein) was used as an endogenous control. The following predesigned TaqMan assays (Life Technologies) were used: TREM2 Hs01010721_m1; TYROBP Hs00182426_m1; TBP Hs00427620_m1; IFNB Hs01077958_s1; IRF7: Hs01014809_g1, IFIH1: Hs00223420_m1.

We designed a pair of custom TaqMan assays which specifically measure the Δe2 transcript (e1/e3: probe spanning exon1/3 junction) vs full-length and other transcripts that retain exon 2 (e2/e3: probe spanning exon 2/3 junction).

Oligo sequences and concentrations for e1/e3 assay were:

forward primer: 5′-TCTTGCACAAGGCACTCT −3′ (600 nM);

reverse primer: 5′- GAACCAGAGATCTCCAGCAT −3′ (600 nM);

probe: 5′- TGTCACAGACCCCCTGGATCACCG −3′ (133 nM).

Oligo sequences and concentrations for e2/e3 assay were:

forward primer: 5′- ACGCTGCGGAATCTACAA −3′ (900 nM);

reverse primer: 5′- GAACCAGAGATCTCCAGCAT −3′ (900 nM);

probe: 5′- CTGGCAGACCCCCTGGATCACCG −3′ (200 nM)

2.7. Western blotting.

Cytoplasmic and plasma membrane fractions were prepared from 6×10^6 cells using the Mem-PER Plus Protein extraction kit (Thermo Fisher, #P-189842). Whole cell lysates or equivalent amounts of fractionated extracts were resolved on polyacrylamide gel, transferred to nitrocellulose membrane and stained with monoclonal TREM2 Abs that recognize C-terminal epitope (Cell Signaling Technology, #91068) at 1:1,000 dilution. For gel loading and cell fractionation controls, we used β-Tubulin (Cell Signaling Technology, #86298, 1:1,000), HRP-conjugated GAPDH (BioLegend, clone W17079A, 1:4,000) and CANX Abs (BioLegend, #699401, 1:2,000). Secondary Abs were: HRP donkey anti-rabbit IgG (BioLegend, #406401, 1:1,000), HRP goat anti-rat IgG (sc-2006, Santa-Cruz, 1:2,000) and HRP goat anti-mouse IgG (Abcam, ab97023, 1:10,000).

2.8. Phagocytosis assay.

In vitro aggregation of amyloid peptide ($A\beta_{1-42}$, Bachem #H-1368, 100 μM in PBS) was carried out by incubation for 4 days at 37°C; insoluble pellet was precipitated and labeled by pHrodo Red according to the manufacturer's protocol (Life Technologies, #P36600). Phagocytosis of aggregated Aβ was measured as described (15). Briefly, THP-1 monocytes were plated at 5×10^5/ml density, TREM2 expression was induced by 100 ng/ml doxycycline overnight (16–18 h). Cells were incubated for 3 h at 37°C with 0.25 μg/ml Aβ-pHrodo that emits a fluorescent signal only in an acidic lysophagosome compartment, collected and washed in ice-cold PBS with 1% BSA. Uptake of Aβ-pHrodo was measured on the LSRII Flow cytometer (BD Biosciences) and data analyzed by FlowJo. 20,000 events per sample were scored, cells were gated by forward and side scatter to include only live cells. Phagocytosis was calculated from mean fluorescence intensity of internalized pHrodo.

2.9. THP-1 differentiation into macrophages and stimulation of IFN I response.

Differentiation to macrophages was performed as previously described (15) using 5ng/ml phorbol 12-myristate 13-acetate (PMA) for 48 h followed by 24 h of recovery without PMA. IFN I response was induced by high molecular weight poly(I:C) complexes with LyoVec transfection reagent (Invivogen, tlrl-piclv, 500 ng/ml), in combination with IFNβ (PeproTech, #300–02, 100 Units/ml) for 24 h. For "add-back" experiments, TREM2 expression was induced by 100 ng/ml doxycycline 16–18 h prior to the IFN I response stimulation.

2.10. Statistical analysis

was performed using Prism version 8 software (GraphPad, La Jolla, CA).

3. Results and Discussion.

We analyzed the distribution of *TREM2* isoforms in human brains using primers to 5′ and 3′ untranslated regions. RT-PCR products were cloned and sequenced. In addition to the annotated isoforms, we identified a novel splice variant lacking exon 2 (Δe2, Fig. 1A) that comprised approximately 40% of sequenced clones (Fig. 1B). Exon 2 skipping produces in-frame deletion of amino acids 14–130 corresponding to a portion of signal peptide and the entire V-set Immunoglobulin (Ig V) domain (aa 29–112, Fig. 1S).

To confirm Δe2 is a naturally occurring *TREM2* isoform, we tested THP-1 and MOLM-13, two cell lines ranked as top TREM2-TYROBP expressors among 1,443 cell lines (https://genevestigator.com, Fig. 1C). Because both PCR and cloning of fragments of different length may introduce an efficiency bias and therefore cannot reliably quantify isoforms, we designed a pair of TaqMan qRT-PCR assays, in which probes spanned either exon 2/3 or exon 1/3 junctions. The e1/e3 assay measures exclusively the Δe2 while e2/e3 assay accounts for all *TREM2* isoforms that retained exon 2. We used plasmids with cloned full-length and Δe2 *TREM2* sequences to verify assay's specificity and to make standard curves for absolute copy number determination. The Δe2 isoform accounted for ~9% and 7% of total *TREM2* transcripts in THP-1 and MOLM-13, respectively (Fig. 1D). In human brain, Δe2 had highly variable expression ranging 3.5–17% in the frontal lobe and 3.7–35% in the hippocampus (Fig. 1F). Both areas expressed comparable levels of total *TREM2* transcript with higher inter-individual variability in the hippocampus (Fig. 1E).

To confirm protein expression of TREM2 isoforms, we used unmodified THP-1 and cells in which exon 2 coding sequence was disrupted by CRISPR/Cas9 (TREM2 KO THP-1, (15)). The editing is expected to terminate translation of all isoforms that retain exon 2 while preserving the Δe2 isoform. Cells were stimulated by IL-4 known to induce TREM2 expression in primary macrophages (20). The membrane was probed with monoclonal antibodies recognizing an epitope at the C-terminus. In unmodified cells, two major bands were observed: the upper one corresponding to full-length protein (predicted size 25 kDa) and the lower one corresponding to the C-terminal TREM2 fragment (CTF, predicted size 8 kDa), a membrane-associated product of proteolytic receptor shedding (Fig. 2A) (21), (22). A minor band of Δe2 polypeptide (predicted size 11 kDa) was located above the CTF band. As expected, TREM2 KO cells did not express full-length protein. Upon IL-4 stimulation, only the Δe2 isoform was increased in TREM2 KO, whereas all TREM2 species were increased in unmodified cells.

Because exon 2 exclusion modifies the signal peptide, we tested if Δe2 was able to sort normally to the plasma membrane. We prepared cytoplasmic and membrane fractions from unmodified THP-1 and THP-1 TREM2 KO cells overexpressing either full-length or Δe2. Equivalent amounts of extracts were resolved on a protein gel and the membrane was probed with Abs recognizing an epitope at the C-terminus. Both endogenous and overexpressed Δe2 proteins were associated with the membrane fraction similarly to full-length and CTF TREM2 (Fig. 2B). The membrane-bound Δe2 underwent further processing to the CTF by receptor shedding proteases, although the shedding efficiency was lower than that of

the full-length form (Fig. 2C). This observation raised a question about turnover of the membrane-bound Δe2. It was previously shown that the membrane-bound CTF undergoes further proteolytic processing by the intramembrane gamma-secretase, an enzyme with high preference to proteins already truncated by receptor shedding proteases (23),(24). Because the Δe2 differs from the CTF by less than 20 residues at the N-terminus, we asked whether the Δe2 may also be a substrate for gamma-secretase. If so, pharmacological inhibition of gamma-secretase will lead to accumulation of Δe2, as well as CTF. After induction of full-length and Δe2 expression, TREM2 KO THP-1 cells were treated with gamma secretase inhibitor DAPT for 24 h (Fig 3A). As expected, CTF level increased in all DAPT-treated samples; neither Δe2 nor full-length TREM2 accumulated upon gamma-secretase inhibition (Fig. 3B-C). Thus, Δe2 is not a substrate of gamma-secretase. To compare efficiencies of full-length and Δe2 conversion to CTF in overexpression experiments, we normalized CTF as percentage of total TREM2 (Fig. 3D). Independently of DAPT treatment, Δe2 processing to CTF was significantly lower than that of full-length indicating that Δe2 is a less than optimal substrate of shedding proteases. The combination of less efficient Δe2 shedding and resistance to gamma-secretase suggests that this isoform might have increased stability, a question that needs to be explored in future studies.

We recently showed that TREM2 ablation substantially blunted IFN I response and reduced Aβ phagocytosis in THP-1 cells; these activities were restored upon overexpression of either common variant or R47H variant proteins (15). To see if the Δe2 isoform complements these deficits, we integrated it into TREM2 KO THP-1 under control of a doxycycline-inducible promoter. Δe2 was unable to restore Aβ phagocytosis in TREM2 KO (Fig. 4A, Fig. 2S); it also had diminished ability to stimulate IFN I response genes *IFNB* and *IFIH1* (Fig. 4B, Fig. 3S). Thus, Δe2, a naturally occurring TREM2 splice isoform enriched in the human brain, does not complement some activities of full-length TREM2.

Alternative RNA splicing is an important mechanism that generates protein diversity by reshuffling functional domains of proteins. Variants that affect splicing regulatory motifs are usually deleterious, and about one-tenth of disease-associated variants reported in the Human Gene Mutation Database are splicing mutations (25). Of known loss of function TREM2 variants responsible for PLOSL and FTD about 20% disrupt the coding frame via altered splicing. These include variants that cause intron 2 retention (26) and exon 3 skipping (27) in FTD or PLOSL patients. A variant in intron 1, c.40+3delAGG, responsible for FTD without bone cysts, weakens the donor splice site resulting in 2-fold reduction of TREM2 level (28).

Exon 2 encodes most of the extracellular TREM2 moiety harboring the AD-associated R47 and R62, as well as the majority of residues mutated in PLOSL and FTD (1). Its absence is likely to affect TREM2 interactions with known ligands and is a plausible cause of Δe2's inability to complement the Aβ phagocytosis deficiency of TREM2 KO cells. Because exon skipping/inclusion are mutually exclusive events, production of the Δe2 transcript is expected to decrease the levels of canonical transcripts that retain exon 2. Additionally, because the Δe2 retains transmembrane and cytoplasmic domains including lysine residue K186 that interacts with D50 in TYROBP (29), this isoform is able to form the complex

with the adaptor protein. Future studies will be required to determine whether Δe2 has an additional regulatory role in TREM2 signaling, for instance by competing for binding with the TYROBP adaptor and sequestering full-length TREM2 from the receptor complex. Data presented in Fig. 3 suggest that a competitive advantage of the Δe2 isoform may be achieved via its less efficient shedding.

TREM2 resides within the TREM gene cluster on chromosome 6p21.1, which also includes structurally similar paralogs TREM1, TREML1, TREML2 and TREML4. Coding and non-coding variants in these genes were found to modify AD risks, independently of TREM2 R47H (30), (31). Interestingly, the TREML1/TLT-1 paralog also has an alternatively spliced transcript (ENST00000437044.2) (32) with skipped exon 2 corresponding to an entire Ig V domain. This suggests that Ig V inclusion/exclusion via alternative splicing is one of nature's tool for functional diversification of some of the Ig-like immune molecules.

The inhibitory CD33 receptor is another microglia-specific AD risk gene (33), (34). Similar to TREM2, CD33 belongs to the superfamily of Ig-like immune receptors, and its Ig V domain is encoded by exon 2 (Fig. 5). Intriguingly, the AD-associated SNP, rs3865444, is in linkage disequilibrium with another SNP in exon 2 (rs12459419). C/T variation in rs12459419 modulates CD33 exon 2 splicing: the AD risk rs12459419-C promotes inclusion of exon 2, while a protective allele, rs12459419-T, instructs exon 2 skipping and production of Δe2 CD33 (35), (36). The full-length CD33 exerts an inhibitory effect on some microglia activities, such as cytokine production and/or phagocytosis in response to AD-relevant stimuli, whereas Δe2 CD33 is unable to suppress microglia activation and amyloid plaque phagocytosis *in vitro* and *in vivo* (37). While a link between alternative splicing and neurodegeneration has been established for CD33, the impact of Ig V domain inclusion/exclusion on TREM2 function and its contribution to neurodegenerative pathologies remain to be elucidated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

PLOSL	Polycystic Lipo-Membranous Osteodysplasia with Sclerosing Leukoencephalopathy
FTD	Frontotemporal dementia
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis

Aβ	Amyloid beta peptide
FL	Full length
CTF	C-terminal fragment
Δe2	exon 2 lacking
IgV	V-set Immunoglobulin
TM	Transmembrane
KO	Knockout
IFN	I Interferon type I

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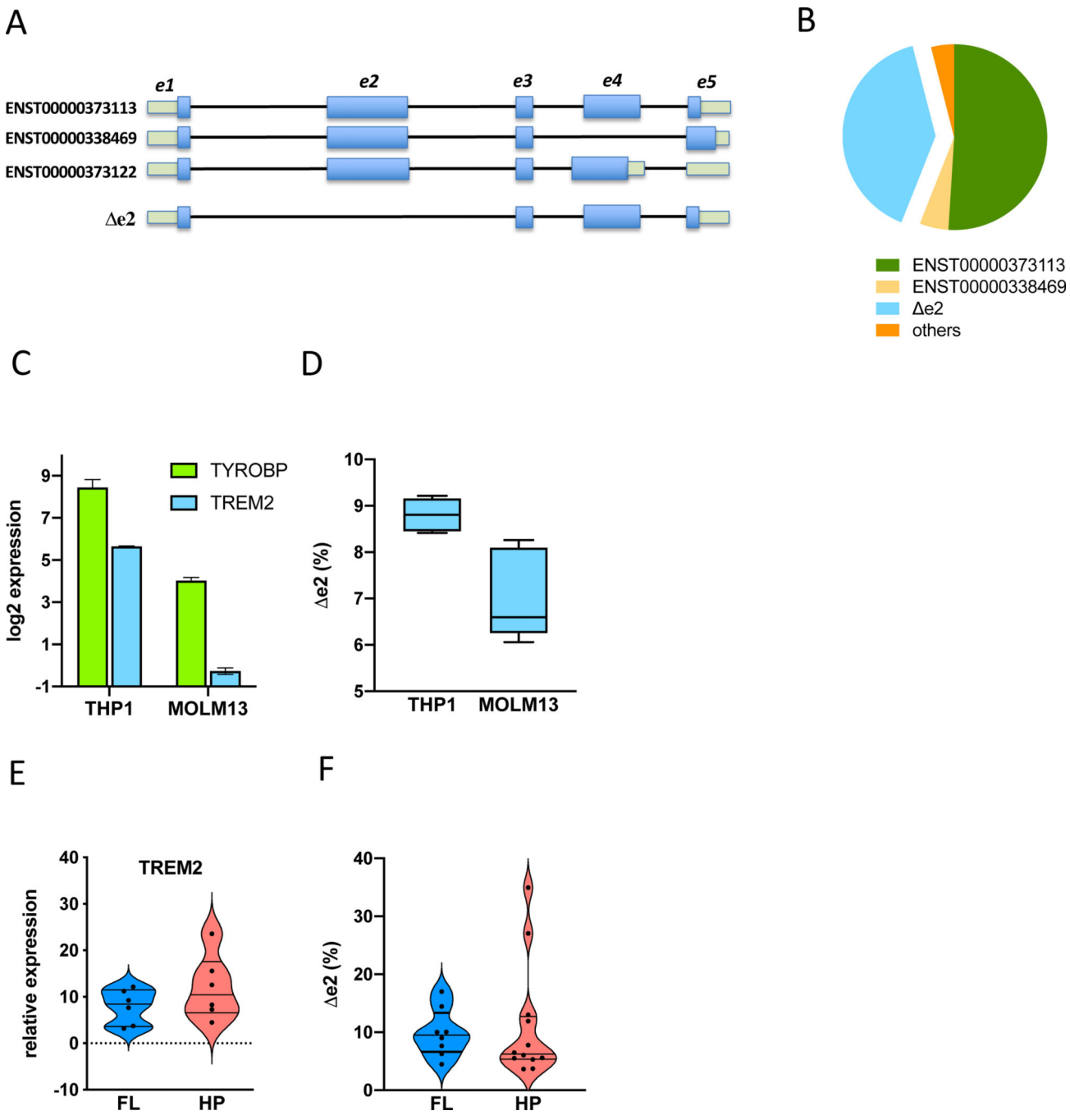

Figure 1. Δe2 ***TREM2*** **splice isoform is enriched in human brain.**

(**A**) Δe2 and three annotated (canonical) alternatively spliced *TREM2* transcripts that retained exon 2. Blue boxes mark coding sequence in exons. (**B**) Frequencies of isoforms in the cDNA library cloned from human frontal cortex (four subjects, N=75 clones sequenced). (**C**) Relative levels of *TREM2* and *TYROBP* transcripts expressed by TREM2-positive THP-1 and MOLM-13 cell lines. (**D**) fraction of Δe2 transcript expressed by these cells. (**E**) Relative levels of *TREM2* expressed in the frontal lobe (FL, N=6) and hippocampus (HP, N=6) of the human brain. Gene expression in C and E was measured using qRT-PCR

with commercial TaqMan assays for *TYROBP* and for major *TREM2* isoforms (specific for e3/e4 junction), expression levels were normalized to *TBP* expression. (**F**) fraction of Δe2 transcript expressed in FL (N=8) and HP (N=12). D, F - Levels of Δe2 and isoforms that retained exon 2 were measured using custom TaqMan assays specific for e1/e3 and e2/e3 junctions, respectively. Absolute copy number of each isoform was calculated using calibration curves of corresponding plasmid standards.

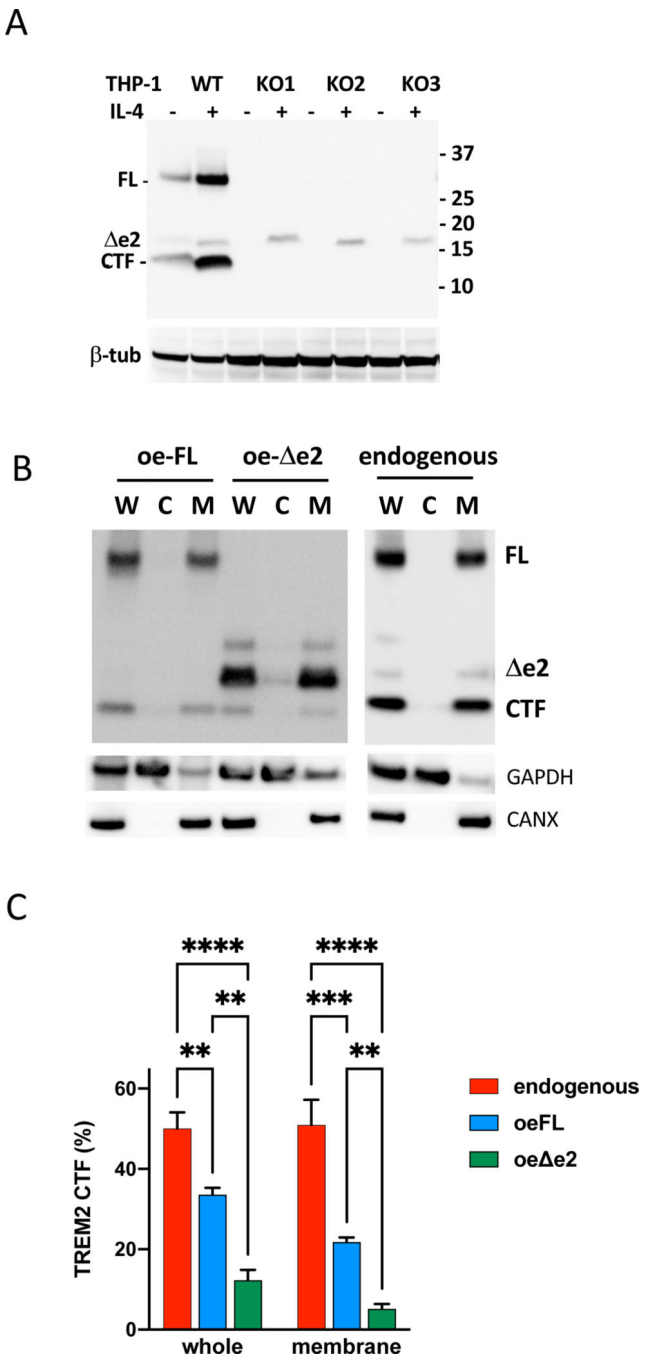

Figure 2. Protein expression and localization of Δe2 TREM2

(**A**). Western blot of whole cell THP-1 extracts probed with anti-TREM2 Abs, after 24 h stimulation with IL-4. WT – unedited cells; KO1-KO3 – independent TREM2 knockout clones. FL – TREM2 receptor encoded by a major canonical isoform; CTF - a membrane-associated C-terminal fragment, a product of full-length receptor shedding due to proteolysis at the amino acids 157–158 (21). Lower strip –loading control probed with anti-tubulin Abs. The experiment was repeated twice on different days; representative western blot is shown. (**B**). Western blot of subcellular THP-1 fractions probed with anti-TREM2 Abs. Left

panel: Overexpression (oe) of full-length (FL) or Δe2 constructs integrated into TREM2 KO THP-1 was induced with doxycycline. Right panel: endogenous TREM2 expressed by unedited THP-1 cells. W – whole cell lysate; C, M - cytoplasmic and membrane fractions, respectively. Strips at the bottom are controls of subcellular fractionation probed with antibodies to GAPDH (cytoplasm) and CANX (membrane). The experiment was repeated twice on different days; representative blot is shown. (**C**) Percentage of CTF calculated from the sum of all TREM2 species. Whole - whole cell lysate, membrane –membrane fraction. Shown are means ± SD of biological replicates; ** - $p<0.01$; *** - $p<0.001$, **** - $p<0.0001$ two-way ANOVA (Tukey's multiple comparison test).

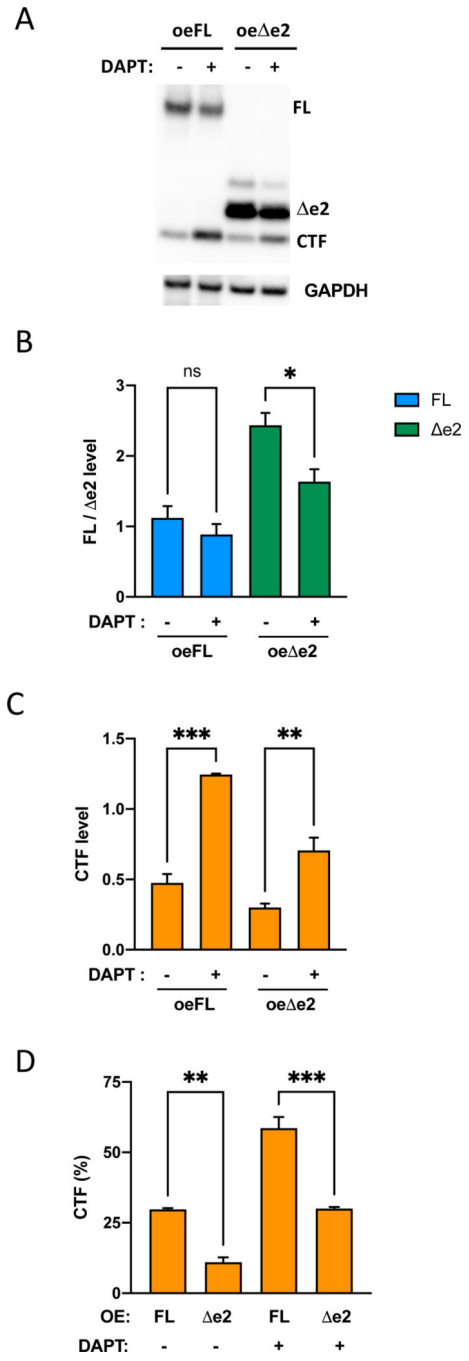

Figure 3. Proteolytic processing of TREM2 isoforms.

(A) A representative western blot of whole cell extracts probed with TREM2 Abs, following 24 h treatment with 10 μM DAPT. TREM2 full-length and Δe2 overexpression (oeFL and oeΔe2) in THP-1 TREM2 KO cells was induced with doxycycline. Lower strip – membrane was probed with HRP-GAPDH Abs. The experiment was repeated twice on different days. (B-D) Quantification of TREM2 protein species normalized to GAPDH expression level. (B) - level of FL and Δe2; (C) – level of CTF; (D) – percentage of CTF. Shown are means ± SD

of biological replicates; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$, one-way ANOVA (Tukey's multiple comparison test).

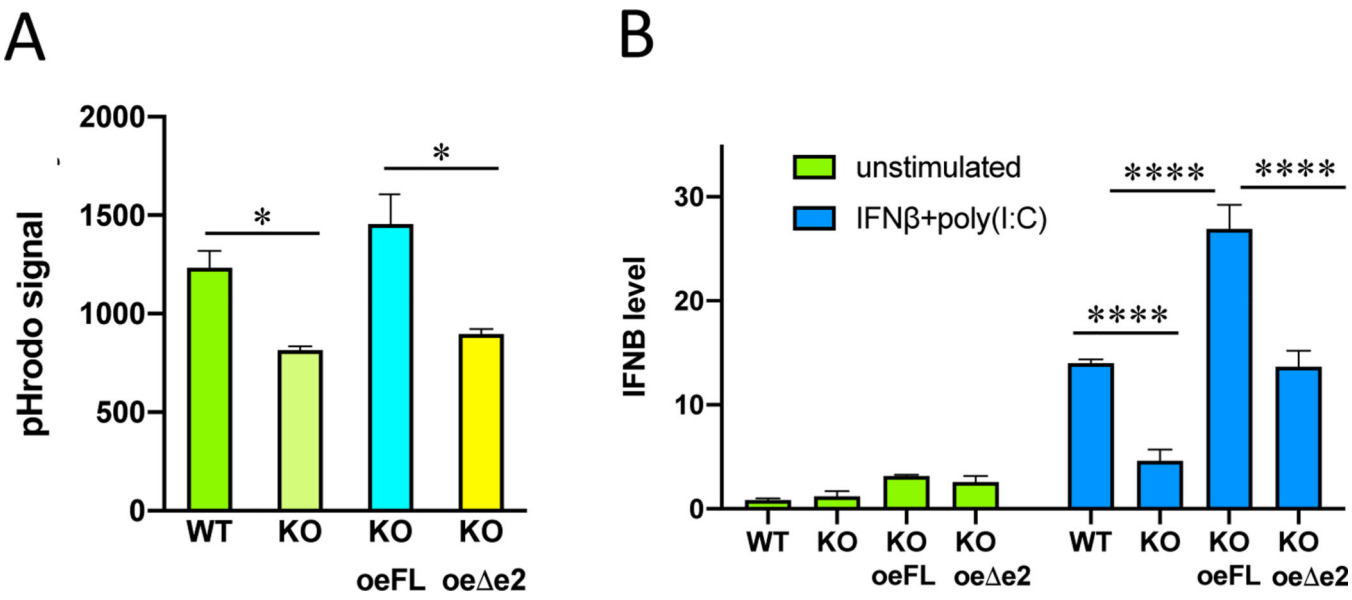

Figure 4. Functional differences of Δe2 and full-length TREM2.

(A) Phagocytosis of aggregated pHrodo-labeled Amyloid beta peptide (Aβ). WT – unmodified THP-1; KO - TREM2 knockout (KO1 clone); oeFL, oeΔe2 – TREM2 KO1 cells expressing full-length or Δe2 isoform, respectively. TREM2 overexpression was induced with doxycycline. Cells were incubated with pHrodo-labeled Aβ and fluorescence measured by flow cytometry. Assays were performed twice; the representative experiment is shown. Shown are means ± SD of biological triplicates; * - $p<0.05$; one-way ANOVA (Dunnett's multiple comparison test). (**B**). IFN I response of *in vitro* differentiated THP-1 macrophages. THP-1 monocytes were differentiated to macrophages by PMA; TREM2 expression was induced with doxycycline prior to stimulation of IFN I response with poly(I:C) + IFNβ. After 24 h stimulation, cells were collected and total RNA isolated. IFN I response was quantified as induction of IFNB mRNA, expression levels were normalized to unstimulated unmodified THP-1. The experiment was repeated three times on different days. WT – unmodified THP-1; KO - TREM2 knockout (KO1 clone); oeFL, oeΔe2 – TREM2 KO1 cells with doxycycline-inducible constructs over-expressing full-length or Δe2 isoform, respectively. Shown are means ± SD of biological triplicates; **** - $p<0.0001$; two-way ANOVA, (Tukey's multiple comparison test).

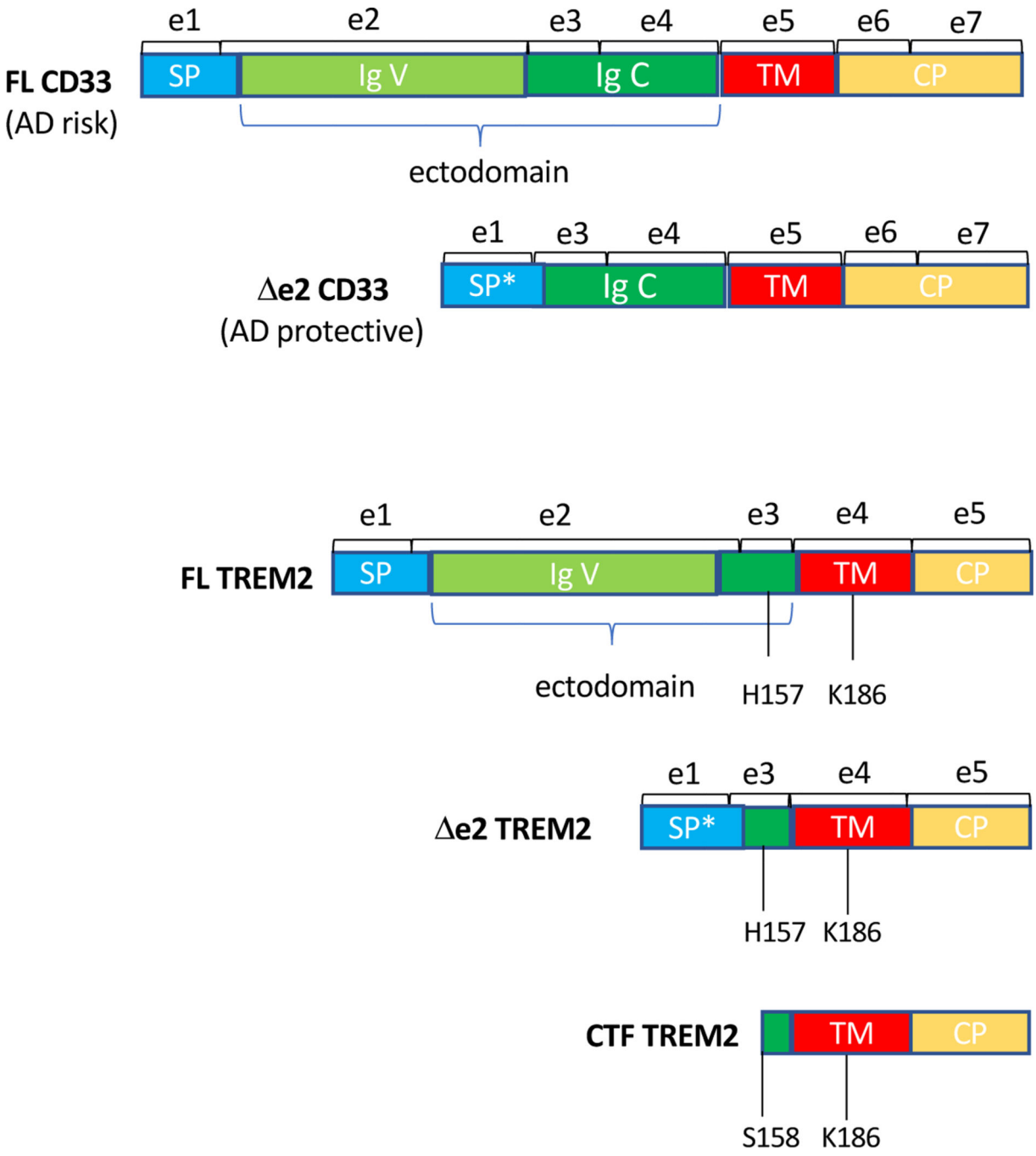

Figure 5. Domain organization of isoforms of microglial receptors TREM2 and CD33.

SP - signal peptide; SP* - signal peptide modified in Δe2; TM - transmembrane domain; CP - cytoplasmic domain; Ig V, Ig C - Immunoglobulin-like domains; FL - full length; CTF - C-terminal fragment, Δe2 - lacking exon 2. H157/S158 - proteolytic shedding site; K186 - conserved residue responsible for interaction with TYROBP adaptor. Corresponding exons are depicted by square brackets. The diagram is not drawn to scale.