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Soluble TREM2 suppresses neuronal activity

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Summary

Neuronal activity must be tightly controlled to maintain brain function. Accumulating evidence identifies microglia as regulators of neuronal activity^{1,2}. The triggering receptor expressed on myeloid cells 2 (TREM2) shapes microglial states³ and is implicated in brain disorders, including Alzheimer's disease (AD)⁴⁻⁸. Cleavage of TREM2 releases its ectodomain, soluble TREM2 (sTREM2)^{7,9}, which has been linked to protective effects¹⁰, but whose binding partners and mechanisms of action remain incompletely understood. Here, we asked whether sTREM2 directly influences neuronal and circuit activity in vivo.

Using mouse models and in vitro systems, we demonstrate that sTREM2 rapidly and reversibly suppresses neuronal activity at both single-cell and circuit levels in the healthy brain. Mechanistically, sTREM2 engages purinergic microglia–neuron signaling² by binding CD73, the ectoenzyme converting AMP to adenosine. Elevated sTREM2 levels protect against kainic-acid (KA) induced epileptic seizures and prevent β -amyloid (A β)-dependent neuronal hyperactivation.

These findings establish a physiological role of sTREM2 as a fast-acting modulator of neuronal activity. In addition, by limiting aberrant circuit activity induced by KA or A β , sTREM2 may protect against excitotoxicity and constitute a potential target for disease-modifying therapies.

Main

The murine triggering receptor expressed on myeloid cells 2 (TREM2) is a 227-amino-acid type I transmembrane receptor expressed in the brain mainly by microglia³. TREM2 is a key regulator of the phagocytosis of apoptotic cells, brain energy homeostasis, synaptic pruning, and the orchestration of the immune response to injury and neurodegeneration^{3,11-14}.

Defective TREM2 interferes with microglia's ability to dynamically react to endo- or exogenous toxins as well as their energy metabolism and phagocytic potential^{8,10,14}. TREM2 loss-of-function results in diseases such as early-onset dementia⁴, while certain variants confer substantial risk for late-onset Alzheimer's disease (AD)^{5,6}.

TREM2 is also increasingly recognized to modulate neuronal circuit function. During development, TREM2-dependent microglial pruning refines synaptic connectivity, and its loss results in hyperconnectivity and increased neuronal excitability^{14,15}. In the adult brain, TREM2 deficiency exacerbates kainic acid-induced seizures¹⁶⁻¹⁸ and impairs long-term potentiation (LTP) in some models^{19,20}, but not in others²¹. Viral overexpression of TREM2 in adult animals reduces spontaneous excitatory postsynaptic currents (EPSCs)²², highlighting the importance of TREM2 for circuit integrity in the mature brain.

Full-length TREM2 (FL-TREM2) is a highly dynamic receptor undergoing rapid turnover at the cell surface with a surface half-life of less than 1h^{7,8}. Cleavage by ADAM10 and ADAM17 leads to the secretion of a 139-amino-acid fragment encompassing residues 19-157, referred to as soluble TREM2 (sTREM2). sTREM2 application can boost cytokine production and promote microglia migration^{23,24}. Still it is unclear, whether and to what extent sTREM2 affects neuronal and circuit activity, especially as studies examining the effects of sTREM2 on LTP have yielded divergent results^{24,25}.

A hallmark of early AD is aberrant neuronal hyperactivity, particularly in hippocampal circuits, which occurs prior to overt neurodegeneration²⁶⁻³⁰. This network dysfunction is closely linked to subclinical epileptiform activity in AD patients³¹. TREM2 signaling is required for the transition of microglia into disease-associated states that enable plaque engagement and containment of neurotoxic A β species^{11,12}. CSF sTREM2 levels rise early during AD progression, and decades before clinical manifestation in familial AD, peak near symptom onset, before decreasing again in symptomatic disease^{10,32,33}.

Here, we ask whether and how sTREM2 affects neuronal activity levels in vivo in the healthy brain and during excitotoxic challenges. We acutely apply recombinant sTREM2 in the hippocampus of wild-type mice and investigate neuronal activity levels in mouse models with altered sTREM2 levels, demonstrating that sTREM2 rapidly downregulates neuronal activity. Mechanistically, sTREM2 acts through a microglia-dependent purinergic pathway, in which ATP released from active synapses is metabolized by CD39 and CD73 into adenosine, suppressing neuronal firing via adenosine A1 receptors (A1R)². We further identify CD73 as a sTREM2 binding partner. At the circuit level, elevated sTREM2 limits kainic acid-induced seizures, whereas reduced sTREM2 increases seizure susceptibility. Finally, experimentally increasing

sTREM2 levels protects against neuronal hyperactivation induced by patient-derived and synthetic A β species.

sTREM2 acutely dampens neuronal activity

To test whether sTREM2 can modulate neuronal activity in vivo, we devised a two-photon calcium imaging experiment, in which we directly applied recombinant sTREM2 to the extracellular space, while monitoring the activity of individual neurons with the organic calcium indicator Cal-520 (Fig. 1b,c). We focused on the hippocampal CA1 region, where sTREM2 has previously been shown to influence plasticity in vitro^{24,25,34}. We expressed the secreted ectodomain of murine TREM2 (amino acids 19–157) with an N-terminal Strep tag in HEK293 cells using a pSec vector encoding a signal peptide (see Document S1) and purified the recombinant sTREM2 from the culture medium (Extended Data Fig. 1a,b). Local application of sTREM2 in CA1 through a glass pipette (pipette concentration: 5 μ M) in 4–6-month-old wild-type mice rapidly reduced spontaneous neuronal activity (Fig. 1c middle, Fig. 1d). After a 10 min washout period, neuronal activity reverted to baseline levels (Fig. 1c, right, Fig. 1d). As a control, we synthesized a sTREM2 loss-of function variant harboring the T66M mutation (sTREM2^{T66M}) linked to fronto-temporal dementia^{4,8} (Extended Data Fig. 1a,b). In contrast to wild-type sTREM2, sTREM2^{T66M} had no effect on neuronal activity (Fig. 1e,f, Extended Data Fig. 1c). Likewise, the application of heat-denatured sTREM2 or vehicle controls was ineffective (Extended Data Fig. 1d-g). As in pipette application experiments the applied substance is strongly diluted with increasing distance from the tip³⁵, we next applied sTREM2 to the circulating artificial cerebrospinal fluid (aCSF) to achieve more uniform tissue distribution. In these bath application experiments, sTREM2 suppressed neuronal activity levels at low nanomolar concentrations (IC₅₀ 5.26 nM, 131.5 ng/mL) (Extended Data Fig. 1h).

Next, we performed electrophysiology experiments at CA3-CA1 Schaffer collateral synapses in acute brain slices to test whether sTREM2 affects synaptic function. Incubation with 50 nM sTREM2 impaired LTP initiated by high-frequency stimulation. In contrast, LTP remained stable in slices treated with sTREM2^{T66M} (Extended Data Fig. 2a,b). Whole-cell patch-clamp recordings from CA1 pyramidal neurons revealed a rundown of EPSCs following bath application of 50 nM sTREM2. Conversely, EPSCs remained stable after sTREM2^{T66M} application (Extended Data Fig. 2c,d). Notably, EPSC amplitude also decreased when

synaptic stimulation was paused after the baseline recording, indicating an activity-independent mechanism (Extended Data Fig. 2e).

As sTREM2 is constitutively shed and maintained at basal levels in the adult wild-type brain ⁷, we asked whether the endogenously present sTREM2 tonically downregulates neuronal activity levels in vivo. To neutralize sTREM2, we used the monoclonal antibody E6T1P targeting the amino-terminal ectodomain shared by both full-length and soluble TREM2 (Fig. 1a). Pipette-application of 5 μ M E6T1P increased neuronal activity (Fig. 1g,h). Unlike the rapid recovery observed after sTREM2 washout, activity levels only partially normalized 10 minutes post-application (Fig. 1h), likely due to sustained neutralization of sTREM2. In contrast, the isotype antibody E7P8J, targeting the intracellular domain of FL-TREM2 inaccessible from the extracellular space, was ineffective (Fig. 1i, Extended Data Fig. 3a,b), as was heat-denatured sTREM2 or sTREM2 preincubated with E6T1P (Extended Data Fig. 3c-h). To exclude that E6T1P also activates FL-TREM2 and its intracellular effectors, we assessed SYK phosphorylation, the main downstream effector of TREM2 ³, in HEK293 cells after 5 minutes of antibody exposure. E6T1P and E7P8J did not induce SYK phosphorylation (Fig. 1j), while the agonistic stalk-binding antibody 4D9 ³⁶ robustly increased it.

sTREM2 substitution reverses the neuronal hyperactivity phenotype of TREM2^{-/-} mice.

To investigate how chronic alterations of sTREM2 levels influence neuronal activity, we analyzed spontaneous neuronal activity levels in the CA1 region of TREM2 knockout (TREM2^{-/-}) mice, which lack both FL-TREM2 and sTREM2. Compared to wild-type controls, these mice exhibited increased neuronal activity (Fig. 2a-e) and a larger proportion of highly active cells, i.e., neurons falling within the highest quartile of the wild-type activity distribution (Fig. 2f).

Considering our previous findings, we suspected that the hyperactivity phenotype of TREM2^{-/-} mice may be explained by a lack of sTREM2. Thus, we directly applied sTREM2 to the extracellular space of the hippocampal CA1 area in TREM2^{-/-} mice while monitoring neuronal calcium dynamics. sTREM2 application suppressed neuronal activity (Fig. 2g, middle, h), and the effect was fully reversible 10 minutes after the application was stopped (Fig. 2g, right, h). During sTREM2 exposure, the activity distribution closely resembled that of untreated wild-type mice (Fig. 2i), indicating that exogenous sTREM2 is sufficient to acutely and reversibly rescue the hyperactive phenotype of TREM2^{-/-} mice. The application of sTREM2^{T66M},

heat-denatured sTREM2 or the E6T1P antibody did not change neuronal activity levels (Fig. 2j-l, Extended Data Fig. 4a-d), ruling out off-target effects of the antibody.

In TREM2^{-/-} mice, increased neuronal activity was not restricted to the hippocampus: pyramidal neurons in layer 2/3 of the frontal cortex showed increased calcium transient frequencies compared to wild-type controls (Extended Data Fig. 5a-d). At the network level, slow-wave synchronization between the frontal and occipital cortex was disrupted in TREM2^{-/-} mice (Extended Data Fig. 5e-g), in line with reduced functional connectivity in this model, as reported previously¹⁵.

Increased sTREM2 levels are associated with lower neuronal activity in TREM2^{H157Y} mice.

Next, we asked whether chronic endogenous overproduction of sTREM2 can also suppress neuronal activity. This can be modelled by introducing a mutation at the cleavage site, leading to higher shedding and constitutively increased brain sTREM2 levels³⁴. We generated a TREM2^{H157Y} knock-in mouse model by exchanging a histidine with a tyrosine at the cleavage site of TREM2 (Fig. 1a) at position 157Y via CRISPR/Cas9 targeting of exon 3 (Fig. 3a, Extended Data Fig. 6a).

Biochemical profiling of 7-month-old homozygous TREM2^{H157Y} and wild-type mice showed no difference in total cellular TREM2 in RIPA lysates (Fig. 3b). In contrast, sTREM2 levels measured in TBS lysates were increased in TREM2^{H157Y} mice (Fig. 3c), accompanied by a reduction of membrane-associated FL-TREM2 (Fig. 3d, Extended Data Fig. 6b). These data demonstrate that the H157Y mutation increases shedding (increased sTREM2:FL-TREM2 ratio) without altering overall TREM2 levels^{7,9,34}, in line with previous data from a similar mouse line³⁴. Although the increase in sTREM2 levels was only moderate (Fig. 3c), TREM2^{H157Y} mice displayed reduced baseline neuronal activity levels in CA1 compared to wild-type mice, characterized by a reduced mean activity, a high number of cells falling into the 1st, and a low number in the 4th quantile of the activity distribution in wild-type mice (Fig. 3e-g, Extended Data Fig. 6c-e).

If this hypoactivity is indeed driven by the genetically determined excess of sTREM2, it should be reversible upon sTREM2 scavenging. To test this, we repeated the E6T1P antibody application in these mice, inducing a significant increase in neuronal activity levels (Fig. 3h, left and middle, Fig. 3i), which was partially reversible 10 minutes post-application (Fig. 3h right, i). Notably, during antibody treatment, neuronal activity in TREM2^{H157Y} mice mimicked that of wild-type mice (Fig. 3j). Again, the E7P8J antibody was ineffective

(Fig. 3k, l, Extended Data Fig. 6f). sTREM2 application did not alter activity levels (Fig. 3m, Extended Data Fig 6g), indicating saturation of sTREM2-mediated activity suppression in TREM2^{H157Y} mice.

Together, acute and genetic manipulations identify extracellular sTREM2 levels as a determinant of basal neuronal activity.

The sTREM2 action requires intact purinergic microglia-neuron signaling

Since microglia are the predominant TREM2-expressing brain cells³, we asked whether they are required for the activity-suppressing effect of sTREM2. We depleted microglia in wild-type mice by administering CSF1R inhibitor-containing chow (PLX5622, 1200 mg/kg) for three weeks (Extended Data Fig. 7a). Consistent with previous reports from other brain regions^{1,2}, spontaneous neuronal activity in hippocampal CA1 of microglia-depleted wild-type mice was markedly elevated compared to control animals (Extended Data Fig. 7b-d). Notably, sTREM2 no longer altered neuronal activity in microglia-depleted wild-type mice (Extended Data Fig. 7e-g), indicating that sTREM2's effect depends on the presence of microglia, rather than a direct interaction of sTREM2 with neurons.

Given the rapid, reversible suppression of neuronal activity by sTREM2 (Figs. 1-3), we hypothesized that it engages a fast microglia-dependent purinergic pathway. In fact, such a pathway was previously described and encompasses the NMDA receptor-dependent neuronal ATP release, P2Y12R-mediated microglial process recruitment, ATP hydrolysis to AMP by microglial CD39, conversion of AMP to adenosine by CD73, and finally A1R-mediated suppression of neuronal activity^{1,2,37} (Fig. 4a).

To test this hypothesis, we first examined the pathway's final effector, A1R^{1,2,38}. Bath application of the A1R antagonist DPCPX significantly increased CA1 neuronal activity (Fig. 4b, Extended Data Fig. 8a), and subsequent sTREM2 application failed to suppress activity (Fig. 4c, Extended Data Fig. 8b), demonstrating that A1R signaling is required for the effect of sTREM2. In contrast, blocking A1Rs failed to further increase neuronal firing in TREM2^{-/-} mice (Extended Data Fig. 8c-d).

We next inhibited P2Y12R, which guides microglial processes expressing ATP-degrading enzymes towards sites of ATP release³⁸. P2Y12R blockage (PSB-0739) abolished sTREM2-induced activity suppression (Extended Data Fig. 8e-f), confirming the requirement for microglial purinergic signaling.

If sTREM2 amplifies A1R-mediated adenosine signaling, its effect should be occluded by the addition of exogenous adenosine. Indeed, the application of a stable adenosine analog (cAdo) reduced neuronal activity (Extended Data Fig. 8g), but co-application with sTREM2 produced no additive suppression (Fig. 4d). Likewise, blocking adenosine uptake with an equilibrative nucleoside transporter (ENT1) inhibitor (dipyridamole) decreased activity (Extended Data Fig. 8h), presumably through extracellular adenosine accumulation, and prevented further suppression by sTREM2 (Extended Data Fig. 8i-j). A1R agonists acutely reduced activity in microglia-depleted or TREM2^{-/-} mice, indicating that downstream A1R signaling remains intact in both models (Extended Data Fig. 8k,l).

To delineate how sTREM2 engages the purinergic signaling cascade, we blocked individual components of the pathway (Fig. 4a). Bath application of an NMDA receptor blocker (APV), inhibitors of CD39 (ARL67156) or CD73 (AOPCP) prevented sTREM2-mediated activity suppression (Fig. 4e, Extended Data Fig. 9a-d) underscoring that an intact purinergic cascade is required.

To identify a molecular interaction partner, we focused on CD73, the terminal adenosine-generating enzyme. Fluorescence polarization measurements using fluorescein-labeled soluble CD73 (sCD73) and sTREM2, revealed a moderate-affinity binding ($K_D = 12.5 \pm 3.8 \mu M$) (Fig. 4f). To assess the functional relevance of this interaction, we engineered a Strep-tagged, catalytically inactive soluble CD73 variant (soluble CD73^{mut}, sCD73^{mut}) lacking the GPI anchor and carrying two active-site mutations (Fig. 4g, Extended Data Fig. 9e). Although enzymatically inactive (Fig. 4h), sCD73^{mut} retained its ability to bind sTREM2 with a similar affinity (K_D of $12.2 \pm 4.3 \mu M$, Extended Data Fig. 9f), suggesting its use as a decoy receptor. Application of sCD73^{mut} to CA1 neurons in TREM2^{H157Y} mice markedly increased neuronal activity (Fig. 4i, Extended Data Fig. 9g), indicating neutralization of elevated endogenous sTREM2. Heat-denatured sCD73^{mut} was inactive (Extended Data Fig. 9h), and sCD73^{mut} had no effect in TREM2^{-/-} mice (Fig. 4j, Extended Data Fig. 9i), demonstrating sTREM2 specificity. Moreover, preincubation of sCD73^{mut} with sTREM2 fully prevented the sTREM2-induced suppression of neuronal activity in wild-type mice (Extended Data Fig. 9j), whereas sCD73^{mut} alone produced only a modest increase (Extended Data Fig. 9k-l).

sTREM2 protects against seizures via A1R agonism in the kainic acid seizure model

Given sTREM2's neuronal activity-suppressing effect, we next asked whether sTREM2 protects against network hyperexcitability and seizures in a kainic acid (KA) challenge model. Following the protocols of Das et al.^{17,18}, we implanted EEG electrodes over the temporal cortex in 2–3-month-old male TREM2^{H157Y} and wild-type mice, and injected KA (10 mg/kg, two injections, 30 min apart) with concurrent EEG recordings (Fig. 5a). TREM2^{H157Y} mice exhibited fewer epileptic spikes than wild-type mice following KA injection, indicating decreased seizure susceptibility (Fig. 5b, c, Extended Data Fig. 10).

In contrast, TREM2^{-/-} mice exhibited more epileptic spikes than wild-type mice after low-dose KA injection (5 mg/kg, two injections 30 min apart) (Fig. 5d-e), consistent with previous reports¹⁶⁻¹⁸. To test whether acute TREM2 depletion in adulthood is sufficient to reproduce this phenotype, we blocked TREM2 expression in wild-type mice using an antisense oligonucleotide (ASO) (Extended Data Fig. 11a, b). One intracerebroventricular injection of the TREM2-ASO lowered total TREM2 in RIPA whole-brain lysates and sTREM2 in TBS extracts compared to control ASO-treated mice after 3 weeks (Extended Data Fig. 11c,d), without altering the protein levels of CD39 or CD73 (Extended Data Fig. 11e). KA-challenged TREM2 knockdown mice responded with a greater number of epileptic spikes than animals injected with a control ASO (Fig. 5 d-e). Overall, spike numbers, behavioral outcomes assessed by Racine score and the relative time spent in electroencephalographically defined seizures of TREM2-knockdown mice mirrored those of TREM2^{-/-} mice, while animals injected with the control-ASO tracked wild-type controls (Fig. 5f; Extended Data Fig. 11f-h).

Given sTREM2's action on CD73 (Fig. 4) and the reduced purinergic tone observed in TREM2^{-/-} mice (Extended Data Fig. 8c, d), we hypothesized that the seizure susceptibility observed in sTREM2-deficient models stems from reduced adenosine-mediated inhibition. Because exogenous adenosine suppressed hippocampal neuronal activity in TREM2^{-/-} mice (Extended Data Fig. 8l), we next examined whether enhancing A1R signaling could rescue their seizure phenotype. Administration of CCPA, a brain-penetrant and selective A1R agonist, normalized seizure susceptibility in KA-challenged TREM2^{-/-} mice, reducing total epileptic spikes and seizure durations to wild-type levels (Fig. 5g-h; Extended Data Fig. 11 i-j). In contrast, CCPA did not alter seizure parameters in KA-challenged wild-type animals, indicating that it specifically compensates for the purinergic deficit caused by sTREM2 loss.

sTREM2 prevents A β -dependent neuronal hyperactivation

Early AD is characterized by emergent neuronal hyperactivity, which is driven by soluble A β dimers and oligomers, and occurs before plaque formation³⁹. This can be modeled by the application of A β dimers directly into the interstitial space surrounding hippocampal neurons of wild-type mice^{26,28}. Given that sTREM2 suppresses neuronal activity and that sTREM2 levels rise early during AD progression^{32,33}, we asked whether elevated sTREM2 can counteract A β -dependent neuronal hyperactivation. To this end, we applied human A β dimers (hA β dimers) isolated from AD brains by size-exclusion chromatography²⁶ into the hippocampal CA1 area of wild-type mice (Fig. 6a, b). Consistent with previous work²⁶, hA β dimers markedly increased the number of calcium transients in surrounding neurons and returned to previous levels after the application was stopped (Fig. 6c, upper row; Extended Data Fig. 12a). Next, we added sTREM2 to the bath solution (10 nM), which reduced ongoing activity (Fig. 6d). Under these conditions, hA β dimers failed to alter neuronal activity levels (Fig. 6c, middle row, Extended Data Fig. 12b). After 30 minutes washout of sTREM2, the ongoing activity recovered, and the application of A β was again able to hyperactivate the same neurons (Fig. 6c lower row, 6d-e, Extended Data Fig. 12c). In contrast, A β monomers (hA β mon) isolated from the same patient did not change neuronal activity levels (Fig. 6f, Extended Data Fig. 12d, e).

To validate this observation, we repeated the experiment using synthetic, disulfide-cross-linked A β dimers [A β S26C]₂⁴⁰. In the same longitudinal experimental paradigm, the application of [A β S26C]₂ (1 μ M) strongly hyperactivated neurons (Fig. 6g, Extended Data Fig. 12f), while in the presence of 10 nM sTREM2, [A β S26C]₂ application was ineffective (Fig. 6g-h, Extended Data Fig. 12g-h). Accordingly, sTREM2 markedly reduced the fraction of highly active neurons during [A β S26C]₂ exposure (Fig. 6i), indicating that elevated extracellular sTREM2 efficiently suppresses the excitatory drive induced by soluble A β dimers.

Together, these findings identify sTREM2 as a potent suppressor of A β -dimer-induced neuronal hyperactivation and suggest that the early rise in extracellular sTREM2 observed during AD may help to stabilize neuronal network activity.

Discussion

sTREM2 is a rapid modulator of neuronal activity

Here, we demonstrate that sTREM2 downregulates neuronal activity in vivo. Genetically determined sTREM2 levels are inversely associated with baseline neuronal activity in hippocampal CA1: TREM2^{-/-} mice exhibit increased neuronal activity, whereas TREM2^{H157Y} mice with elevated sTREM2 show reduced activity compared to wild-type controls. sTREM2 application rapidly suppresses neuronal activity in wild-type and TREM2^{-/-}, but not in TREM2^{H157Y} mice, consistent with a ceiling effect in the latter model. Conversely, antibody-mediated scavenging of endogenous sTREM2 increases neuronal activity in TREM2^{H157Y} and wild-type mice, indicating that endogenous sTREM2 levels tonically restrain neuronal activity, but is ineffective in TREM2^{-/-} animals. This relationship is extended to pathological network activity: TREM2^{H157Y} mice are protected against kainic-acid-induced epileptiform activity and seizures, whereas ASO-mediated knockdown of TREM2, which reduces sTREM2, exacerbates seizure susceptibility. Together, these data establish sTREM2 as a key regulator of neuronal excitability and suggest that the reduced seizure threshold observed in TREM2^{-/-} models¹⁶⁻¹⁸ may partly be attributable to the absence of sTREM2.

Several observations indicate that these effects are mediated primarily by shed sTREM2 rather than membrane-bound FL-TREM2. First, acute sTREM2 application suppressed neuronal activity in TREM2^{-/-} mice, establishing that neither FL-TREM2 nor its downstream signaling is required for this effect. Second, sTREM2 and FL-TREM2 abundance were functionally dissociated across models. TREM2^{H157Y} and TREM2^{-/-} mice both have reduced FL-TREM2 but exhibit opposite sTREM2 levels and neuronal phenotypes, while ASO-mediated reduction of both protein species increased seizure susceptibility. Thus, neuronal activity tracks more closely with sTREM2 than with FL-TREM2 abundance. Third, whereas established FL-TREM2-dependent responses generally unfold over longer timescales³, sTREM2 altered neuronal activity within minutes. TREM2 shedding, therefore, not only terminates receptor signaling but also generates a biologically active molecule that extends TREM2-associated protective functions to the rapid control of network excitability. Although additional contributions from FL-TREM2 cannot be excluded, our findings identify sTREM2 as the main determinant of neuronal activity in vivo.

A physiological role for sTREM2 is further supported by the efficient shedding and rapid turnover of FL-TREM2^{7,8}, its reported effects on synaptic plasticity²⁵, and the growing number of proposed interaction partners¹⁰. An alternatively spliced soluble isoform, sTREM2²¹⁹, is secreted independent of proteolysis^{41,42}. However, its physiological relevance remains unclear given its low concentration in human CSF relative to shed sTREM2⁴¹.

Although the concentrations applied here exceeded reported human CSF levels⁴³, local sTREM2 concentrations may be substantially higher, particularly after injury. In addition, effective tissue concentration rapidly declines with distance from the application pipette in vivo³⁵. Notably, neuronal activity is already reduced in TREM2^{H157Y} mice despite only a modest elevation in sTREM2, supporting the physiological relevance of this mechanism.

Mechanistically, we identify that, rather than acting directly on neurons, sTREM2 suppresses neuronal activity by modulating purinergic signaling. Microglia dynamically respond to changes in neuronal activity, dampening hyperactivity². Strong glutamatergic activity triggers ATP release and recruitment of microglial processes through P2Y12R signaling^{38,44,45}. ATP is subsequently converted to adenosine by the CD39-CD73 pathway, resulting in A1R-dependent suppression of neuronal excitation and seizures^{2,46} (Fig. 4a). Because sTREM2's suppressive effect was absent in microglia-depleted mice and occluded by adenosine, our data indicates that sTREM2 stabilizes a tonic purinergic tone on neuronal activity rather than directly interacting with neuronal receptors. We further identify CD73 as a candidate binding partner in in vitro experiments and establish a decoy receptor function of catalytically inactive sCD73 in vivo. The rapid actions of sTREM2 may complement longer-term FL-TREM2-dependent regulation of network excitability through synaptic remodeling and pruning^{15,47}. Together, these findings indicate that membrane-bound TREM2 and its soluble ectodomain may act across distinct timescales to stabilize neuronal circuits and prevent hyperexcitability.

Implications for brain diseases

The suppressing effect of sTREM2 on neuronal activity has broad implications for brain disorders in which TREM2 expression and shedding are dynamically regulated in response to pathology.

316 In our A β -application model, elevated sTREM2 levels protect CA1 neurons from hyperactivation induced
317 by A β dimers. The early neuronal hyperactivity observed in AD patients and mouse models of β -amyloidosis
318 results from A β dimer- and oligomer-induced glutamate accumulation and excessive excitatory drive ²⁶⁻²⁹
319 together with impaired inhibition ³⁰. Thus, the acute application of A β dimers into the extracellular space of
320 wild-type mice models an early pathogenic state ^{26,28}, in which diffusible A β species accumulate before
321 significant plaque formation. Here, we found that high sTREM2 levels protect against hyperactivation
322 induced by both synthetic, and human AD brain-derived A β dimers ²⁶.

323 sTREM2-dependent suppression of neuronal hyperactivation may therefore be relevant during
324 presymptomatic AD stages. CSF sTREM2 levels rise early on and peak near symptom onset ^{10,32}. In
325 autosomal-dominant AD, CSF sTREM2 levels increase up to five years before clinical manifestation ³².
326 Importantly, patients with high CSF sTREM2 levels ^{10,33} exhibit delayed disease onsets and slower cognitive
327 decline. Traditionally, CSF sTREM2 levels have been interpreted as a surrogate marker of microglial
328 activation and of the abundance of signaling competent FL-TREM2 at the cell surface. In fact, multiple lines
329 of evidence suggest that FL-TREM2 mediates protective signaling at early AD stages ^{24,34,47,48}. Our
330 observation that sTREM2 itself may play a decisive protective role, shielding neurons from A β -dependent
331 hyperactivation, provides further support for the beneficial roles of TREM2 during the early stages of
332 Alzheimer's pathogenesis.

333 Our findings suggest that sTREM2 is not merely a byproduct or biomarker of TREM2 activity, but an active
334 modulator of neuronal network excitability. In addition to its effects on neuronal activity, sTREM2 could
335 mediate alterations in purinergic signaling in the early stages of amyloidosis ⁴⁹. Moreover, sTREM2 can
336 bind A β species, limiting their aggregation, or promoting their clearance, and reducing neurotoxicity ^{24,50,51}.
337 In addition, sTREM2 fragments can modulate complement-dependent synapse loss in tauopathy models
338 ⁵². At the same time, the identification of H157Y as a risk variant for late-onset AD ⁵³ indicates that increased
339 sTREM2 alone is not sufficient for protection, particularly when signaling competent FL-TREM2 at the
340 microglial membrane is reduced^{9,11}.

341 Our results also have important implications for therapeutic targeting of TREM2 in AD. Several agonistic
342 monoclonal anti-TREM2 antibodies are currently being evaluated for their ability to stimulate protective

TREM2-dependent microglial functions ¹³. A common feature of these antibodies is that they bind both FL-TREM2 and sTREM2, yet their impact on sTREM2 function is unknown. Dampened neuronal activity resulting from increased sTREM2 levels suggests potentially beneficial therapeutic outcome if agonistic antibodies not only activate cell autonomous signaling in microglia but additionally lead to increased sTREM2 availability ⁵⁴.

Beyond neurodegenerative diseases, TREM2 also plays an important role in regulating microglial responses in other brain disorders including ischemic stroke ⁵⁵ and glioblastoma ⁵⁶. In these conditions, sTREM2 may help dampen neuronal activity, partially mitigating excitotoxicity or even activity-mediated tumor growth ⁵⁶.

In summary, our findings warrant further investigation of sTREM2's actions in neurodegenerative and other brain disorders, where increased sTREM2 levels may help rectify hyperexcitable networks. In addition, our data highlight that sTREM2, far from being a byproduct, is an important mediator of circuit function, a factor that should be carefully considered in the design and timing of TREM2-targeted therapies ¹³.

Methods

Mice

Housing and maintenance of mice

All surgical and experimental procedures were performed in compliance with ethical regulations and explicitly approved by the government of Upper Bavaria. Mice were housed in standard cages with a 12-hour light/dark cycle, controlled temperature (~25 °C), and stable humidity (~55%). Food and water were available ad libitum.

Mouse lines, age and sex

In vivo two-photon experiments used male and female wild-type C57BL/6J (Charles River), homozygous TREM2 knockout (TREM2^{-/-}, C57BL/6J-Trem2^{em2Adiuj}/J; provided by Marco Colonna⁵⁷), and homozygous TREM2^{H157Y} (C57BL/6J-Trem2^{em3Bwef}/J) mice generated in-house (see below) aged 4–6 months. Slow-wave experiments used both sexes aged 2–3 months, whereas acute brain-slice experiments used wild-type mice aged 4–8 weeks, with male mice used exclusively for patch-clamp recordings. EEG experiments used males aged 2–6 months. Mice were randomly assigned to experimental groups. For CSF1R inhibition, mice received chow containing 1200 mg/kg PLX5622 ad libitum for 3 weeks before experimentation.

Generation of TREM2^{H157Y} knock-in mice

CRISPR/Cas9-mediated genome editing in C57BL/6J zygotes⁵⁸ was used to generate TREM2^{H157Y} knock-in mice. Pronuclear-stage zygotes derived from C57BL/6J matings were microinjected with a TREM2-specific CRISPR/Cas9 ribonucleoprotein complex consisting of 50 ng/μl recombinant SpCas9 protein (IDT), 0.6 μM crRNA (IDT), 0.6 μM tracrRNA (IDT), and 25 ng/μl mutagenic single-stranded oligodeoxynucleotide (ssODN) ssTrem2-H157Y donor template encoding the p.H157Y (CAC>TAC) substitution together with two silent mutations to disrupt the protospacer and introduce a diagnostic Alw44I restriction site. Injected zygotes were transferred into pseudopregnant CD-1 foster females. Founder animals were crossed to C57BL/6J mice, and germline transmission was confirmed in F1 offspring by restriction fragment length polymorphism analysis and Sanger sequencing of genomic DNA. Potential off-target sites predicted using

CRISPOR⁵⁹ were PCR-amplified and sequenced using the primers listed in Document S1. Animals without detectable off-target modifications were used for further breeding.

Genotyping of TREM2^{H157Y} mice

Genomic DNA from ear biopsies was obtained by proteinase K digestion overnight at 55°C (all in mM: 200 NaCl, 100 Tris (pH 8.3), 5 EDTA, 0.2% SDS, 500 µg/mL proteinase K). Lysates were diluted 1:100 in water and 4 µL were used for PCR amplification of the targeted TREM2 locus using GoTaq G2 DNA polymerase (Promega). PCR products were digested with Alw44I for 4 h at 37°C and resolved on 1.5% agarose gels stained with GelRed.

Protein extraction from whole brain powder and (s)TREM2 ELISA

Protein extraction from whole brain powder was carried out as reported previously⁶⁰ using TBS buffer. Levels of soluble TREM2 (sTREM2) in TBS fractions and total TREM2 in RIPA fractions were quantified by MSD ELISA as described in^{60,61}, loading 20–30 µg total protein per well.

Cell culture and protein generation

Cloning of constructs for protein expression

cDNA constructs (see Document S1) were generated from in-house plasmids⁸ or purchased vectors (Origene, CD39, CD73). Target sequences were amplified by PCR using the primers listed in Document S1, digested with the indicated restriction enzymes, and subcloned into pSEC-Zeo 2.1 for the expression of soluble proteins. For the expression of CD39/CD73 full-length proteins Origene pCMV6 entry vectors with an empty vector as control were used for transfection.

Cell transfection and sample harvesting

HEK293-T cells (Invitrogen) were cultured in Dulbecco's modified Eagle's medium with Glutamax (DMEM, Invitrogen), supplemented with 10% fetal calf serum (FCS, Invitrogen). Subconfluent dishes were transfected either transiently with Lipofectamine 2000 (ThermoFisher Scientific) or stably using Zeocin (ThermoFisher Scientific) for selection. In case of transient transfection, cells were transfected overnight in 10 cm dishes using 5 µg of plasmid. Empty vectors were used as a control. Next day, medium was replaced with standard DMEM supplemented with 1x NEAA, Penicillin/Streptomycin and 10% FCS (DMEM F10). After 6 hrs, the medium was refreshed with 5 ml of serum-free OPTIMEM (ThermoFisher Scientific).

Conditioned medium was collected after 20 h and cleared by centrifugation (6000 rpm, 5 min, 4°C). Cells were washed with PBS, harvested by scraping, pelleted (6000 rpm, 5 min, 4°C), and stored at -80°C. HEK293 Flp-In (Life Technologies) cell lines stably overexpressing HA-TREM2 and DAP12-V5 were generated and maintained as previously described³⁶.

Protein purification and quality control

Zeocin-resistant clones were expanded in large culture dishes (24 cm) in DMEM F10 containing Zeocin. At full confluency, medium was replaced with OPTIMEM (20 mL/plate). Conditioned medium was collected after 24 h and immediately filtered (0.22 µm, Merck Bioscience). 0.5–1 L of conditioned medium was subjected to affinity purification using Strep-Tactin®XT 4Flow® high-capacity cartridge and corresponding buffers (IBA Lifesciences, Cat# 2-5027-001) on an ÄKTA FPLC system according to the manufacturer's instructions. Purified fractions were pooled and buffer-exchanged into aCSF (all in mM: 119 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1.3 MgSO₄, 2.5 CaCl₂, 11 glucose) using Amicon Ultra centrifugal filters (10 kDa MWCO, Merck Sigma-Aldrich) with four wash cycles at 4°C.

Protein concentration was determined by NanoDrop spectrophotometry using calculated extinction coefficients (see Document S1). Purified proteins (0.5–2 µg) were resolved by Tris-Glycine SDS-PAGE and stained with Coomassie Brilliant Blue G-250. Gels were imaged using the Gelstick Imager system (Intas).

Western Blots

Conditioned media or RIPA cell lysates (20–30 µg total protein per lane) were separated on 8–12% Tris-glycine (TG) SDS-PAGE gels and transferred onto PVDF (pre-equilibrated in isopropanol) or nitrocellulose membranes. Nitrocellulose membranes were boiled in PBS for 5 min before blocking. Membranes were blocked for ≥45 min in TBS (PVDF) or PBS (nitrocellulose) and incubated overnight at 4°C with rat anti-TREM2 5F4 (in-house), rabbit anti-TREM2 (Abcam, Cat# ab305103), rabbit anti-CD73 (Cell Signaling Technology, Cat# 13160S), rabbit anti-CD39 (Thermo Fisher Scientific, Cat# PA5-120870), anti-β-actin (Sigma-Aldrich/Merck, Cat# A2228), or anti-Calnexin (Enzo Life Sciences, Cat# AD-SPA-860). After washing in TBS-T or PBS-T, respectively, membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature and developed by enhanced chemiluminescence (Amersham). Signals were detected by exposure to X-ray film and developed using a Cawomat 2000 IR system. Western

Bright ECL (Advansta) was used for detection of full-length TREM2 in brain lysates from TREM2^{H157Y} mice. For detection of patient-derived A β in size-exclusion chromatography fractions, western blotting was performed using mouse anti- β -amyloid 17–24 clone 4G8 (BioLegend, Cat# 800708).

p-SYK AlphaLISA

Phospho-SYK (p-SYK) levels following antibody-mediated activation of TREM2 signaling were quantified by AlphaLISA in lysates from HEK293 cells (ATCC, Cat# CRL-1573) stably expressing murine TREM2 and DAP12 as previously described³⁶. Cells were stimulated with the TREM2-activating antibody 4D9 (see³⁶), and antibodies targeting the N- and C-terminus of TREM2 (E6T1P Cat#61788 and E7P8J #76765, Cell Signaling Technology).

Immunohistochemistry

For immunohistochemical analysis, mice were perfused intracardially with ice-cold 4% paraformaldehyde (PFA), and brains were post-fixed overnight at 4 °C. Brains were sectioned into 70 μ m-thick slices and stored in cryoprotectant solution until further use. For Iba-1 staining, sections were washed in PBS, blocked for 90 minutes in 5% donkey serum containing 0.5% Triton X-100 and 1% bovine serum albumin, incubated for three nights at 4 °C with rabbit anti-Iba-1 (1:200; Invitrogen, Cat# PA5-27436) and stained with a donkey anti-rabbit secondary antibody (1:500; Invitrogen, Cat# A-21206) for 2 hours at room temperature and DAPI (1:1000) for 5 minutes. Sections were mounted in Mowiol 4-88 (Electron Microscopy Sciences, Cat# 17977-150) mixed with AF300 (1:10; Electron Microscopy Sciences, Cat# 17977-25). Imaging was performed using a confocal microscope (Olympus) with a 4X or 20X objective.

In vivo fluorescence imaging

Two-photon calcium imaging

In vivo experiments were performed according to previously established procedures^{26,27}. Briefly, mice were anesthetized with isoflurane (2% vol/vol in O₂) and a cranial window (~2 mm) was prepared above hippocampal CA1 (AP -2.75 mm, ML 3.5 mm). A recording chamber was attached to the head, superfused with 37°C artificial cerebrospinal fluid (aCSF; in mM: 125 NaCl, 4.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 20 glucose; pH 7.4 when bubbled with 95% O₂/5% CO₂) and the cortex above the hippocampus removed by suction. The calcium indicator Cal-520 (Bioquest, Cat# 21130) was locally

injected into the imaging region to label neurons and subsequently record spontaneous calcium transients. Antibodies and drugs were stored according to the manufacturers' instructions and diluted to the final working concentration in aCSF immediately prior to use. Proteins and antibodies used as heat-denatured controls were incubated at 70 °C for 1–2 hours before application.

For the localized application of anti-TREM2 E6T1P (Cell Signaling Technology, Cat #61788), antiTREM2 E7P8J (Cell Signaling Technology, Cat #76765), cAdo (Hycultec, Cat# HY-W008344), adenosine (Sigma-Aldrich, Cat# 58-61-7), [A β S26C]₂ (Bachem, Cat# 4089807) or human A β peptides (see below), a glass patch pipette (tip resistance 3-5 M Ω) was filled with ~1 μ L of the drug or protein solution. The pipette was positioned next to the imaged cells and substances were pressure-applied (40-60 mbar, 4-5 min) until a slight displacement of the surrounding tissue confirmed successful injection. The same field of view was maintained throughout baseline, application, and washout phases, with the pipette left in place. Cells lost due to drifts of the field of view during substance application were excluded from the analysis. Neuronal resting activity was recorded for at least 5 min per neuron.

For bath application experiments, substances (APV (Tocris Bioscience, Cat# 0106), ARL67156 (Tocris Bioscience, Cat# 1283), AOPCP (Sigma-Aldrich, Cat# 3768-14-7), DPCPX (Tocris Bioscience, Cat# 102146-07-6), PSB-0739 (Tocris Bioscience, Cat# 3983), or dipyridamole (Sigma-Aldrich, Cat# 58-32-2)) were added to the circulating aCSF following baseline acquisition. Wash-in recordings were performed 10–20 min after substance addition. Wash-out recordings were performed 30 minutes after substance removal.

Two-photon imaging was carried out using a custom-built multiphoton imaging system based on an upright BX51WI microscope (Olympus). Excitation at 920 nm was provided by a tunable, pulsed Ti:Sapphire laser (Coherent), directed through a resonant galvanometric scanner (8 or 12 kHz, GSI Group), at a frame rate of 40 Hz. A 40 $\times$, 0.8 NA water-immersion objective (Nikon, Tokyo, Japan) focused the beam onto the sample in aCSF.

Slow wave fiber fluorometry

Measurements of slow-wave oscillations were conducted as described previously⁶², using OGB-1 AM (ThermoFisher, Cat #O6807) bolus loading in the frontal and occipital cortex. Recordings were acquired using either a sCMOS camera system (Andor Zyla 4.2, Oxford Instruments) at 14.3 Hz or via two implanted

multimode optical fibers (200 μm , 0.48 NA; Thorlabs) coupled to an avalanche photodiode, sampled at 2 kHz. Excitation was delivered at 488 nm by a laser (Coherent) or 450 nm by a photodiode (Thorlabs).

Analysis

Offline analysis of calcium imaging data was performed in MATLAB (MathWorks Inc) using a custom-written toolbox. Image sequences were registered using a rigid-body algorithm, neurons were segmented using MATLAB's implementation of Cellpose using the cyto2 model⁶³ and regions of interest (ROIs) were drawn around individual somata. Each ROI was visually inspected, and its location was manually corrected as necessary. Astrocytes were excluded from the analysis based on their distinct morphology.

Fluorescence signals from each ROI were extracted over time, baseline-corrected where bleaching was apparent, smoothed using a Savitzky-Golay filter (window length = 5 frames), and normalized to baseline fluorescence as $\Delta F/F = (f(t) - f_0) / f_0$, where f_0 was defined as the 10th percentile of the entire fluorescence trace. To detect calcium transients, the resulting traces were deconvolved using OASIS⁶⁴ with a double-exponential kernel (rise time: 20 ms, decay time: 500 ms), and transients were identified as events with peak amplitudes at least four times the standard deviation of the baseline and a minimum prominence of 0.5 in the inferred activity. Activity distributions were visualized using kernel density estimation (ksdensity in MATLAB) with 200 evaluation points. Neurons were classified as low or high activity based on quartile cutoffs from wild-type recordings: $< 1.2 \text{ Ca}^{2+}$ transients/min (lowest 25%) and $> 6.4 \text{ Ca}^{2+}$ transients/min (highest 25%). Calcium transients recorded during the application phase were normalized to baseline activity recorded prior to application. For the dose-response analysis of sTREM2, a four-parameter logistic function was fitted to the averaged, normalized calcium transients using a non-linear least squares method.

Fluorometry calcium traces from frontal and occipital regions were low-pass filtered at 20 Hz, and $\Delta F/F$ was calculated using the 10th percentile of the signal as f_0 . Pearson correlation coefficients were computed between the two traces in 30-second segments, and a mean value was derived per mouse.

Electrophysiological recordings in acute brain slices

Acute brain slices were prepared as previously described^{65,66}. For LTP-recordings, the brains were sectioned into 300 μm horizontal slices in ice-cold carbonate-buffered sucrose-based cutting solution (all in mM: 20 PIPES, 200 sucrose, 10 glucose, 2.5 KCl, 0.5 CaCl_2 , 10 MgSO_4 , and 1.25 NaH_2PO_4). Slices

recovered for ≥ 1 h at room temperature in recovery solution. For patch-clamp experiments⁶⁶, 250 μm slices were prepared using a carbonate-buffered sucrose cutting solution (all in mM: 234 sucrose, 2.5 KCl, 1.25 NaH_2PO_4 , 10 MgSO_4 , 0.5 CaCl_2 , 26 NaHCO_3 , and 11 glucose, pH 7.4) and additionally recovered for 1 h at 37°C in aCSF (all in mM: 119 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 26 NaHCO_3 , 1.3 MgSO_4 , 2.5 CaCl_2 and 11 D-glucose) before recording.

LTP recordings at Schaffer collateral-CA1 synapses were recorded using an isolated pulse stimulator (A-M Systems) and an EXT-10-2F amplifier (npi electronic GmbH). Briefly, fEPSPs were recorded during stimulation (0.1 ms, 20-150 μA , 0.05 Hz) and LTP was induced after a stable 30 min baseline with 100 pulses at 100 Hz with subsequent monitoring for ≥ 75 min. fEPSP rising slope was calculated by least-squares fitting over 1 ms and normalized to the final 10 min of baseline.

Whole-cell patch-clamp recordings were performed in an upright microscope (SliceScope, Scientifica Ltd) using a Multiclamp 700B amplifier (Molecular Devices), controlled via pClamp10 software (Molecular Devices). Slices were continuously superfused with aCSF at $31 \pm 1^\circ\text{C}$. Patch pipettes (5–6 $\text{M}\Omega$) were filled with internal solution (all in mM: 117.5 CsMeSO_4 , 15.5 CsCl , 10 TEA-Cl , 8 NaCl , 0.25 EGTA, 10 HEPES, 4 Mg-ATP , and 0.3 Na-GTP , pH 7.3, 290–295 mOsm). EPSCs were recorded at a holding potential of -65 mV and recordings were excluded if either parameter changed by more than 20%. Schaffer collaterals were stimulated with an aCSF-filled glass pipette (0.0667 Hz, 0.1 ms, 10 ± 5 μA). Two stimulation paradigms were used: (1) continuous stimulation during an 8-min baseline and 30-min protein application, or (2) stimulation during a 5-min baseline and the final 5 min after a 15-min unstimulated peptide incubation. sTREM2 and sTREM2^{T66M} (50 nM final concentration) were bath-applied via the circulating aCSF and were interleaved within the experimental day.

Data was analyzed using Clampfit 10 (Molecular Devices). For time-course analysis, EPSC amplitudes were averaged in 1-min bins and normalized to the final 5 min of the baseline period. The effects of sTREM2 and sTREM2^{T66M} were quantified by normalizing responses after wash-in to their respective baseline conditions.

Fluorescence polarization for sCD73-sTREM2 binding interactions

Purified sCD73 was labeled with a threefold molar excess of NHS-fluorescein (Thermo Fisher Scientific) in a buffer (all in mM: 20 HEPES (pH 7.6), 150 NaCl) and incubated overnight at 4°C. Unreacted dye was removed by size-exclusion chromatography using the same buffer. Fluorescence polarization measurements were carried out at 25°C using an FP-8300 spectrofluorometer (Jasco) equipped with a temperature-controlled water bath (MCM-100). Labeled sCD73 was measured in a 10x2 mm quartz cuvette with excitation at 494 nm and emission at 518 nm. Increasing concentrations of unlabeled sTREM2 in the same buffer were titrated into the cuvette, and changes in fluorescence polarization were recorded. K_D values were derived by fitting the data to a one-site binding model using the Morrison equation.

CD73 activity assay

CD73 enzymatic activity was assessed using a colorimetric CD73 activity assay kit (BPS Bioscience, Cat# 72058) according to the manufacturer's protocol. Purified recombinant sCD73 or sCD73^{mut} was incubated with AMP substrate in assay buffer, with CD73 inhibitor PSB-12379 (Tocris Bioscience, Cat# 6083) as control. Product formation was quantified by measuring absorbance at 563 nm using a plate reader (PowerWave XS, BioTek).

Video-EEG recordings

Surgery

EEG implants were assembled by soldering silver wire electrodes (0.125 mm diameter; Degussa, silver 999) to a multichannel socket and plug connector (RS Components, Cat# 681-3032 and Cat# 681-3017). Mice were anesthetized, and electrodes (temporal: AP +2.0 mm, ML +2.0 mm, reference: AP -1.0 mm, ML -1.0 mm) were placed between the skull and dura. Two electrodes were implanted in the neck muscles for EMG recordings. Electrodes and connectors were secured with dental cement (Ivoclar vivadent, Cat# 634849). Mice were allowed to recover for at least one week before recordings.

EEG and video monitoring were conducted in a recording chamber with one transparent wall, utilizing a custom MATLAB application. The electrode socket was connected to a commutator mounted on a rotatable, weight-balanced swivel to allow free movement. Signals were amplified 1000x, bandpass filtered (0.1–500 Hz; EXT-16DX, npi electronic GmbH), and digitized at 1000 Hz. The video was recorded at 24 Hz using a webcam (Lenovo). After a 1-hour habituation period, a 1-hour baseline EEG was recorded. Kainic acid (5

or 10 mg/kg body weight, diluted in 0.9% NaCl; Tocris Bioscience Cat# 7065) was injected subcutaneously (two injections, 30 min apart). Video-EEG was recorded for 5 hours after injection. CCPA (3 mg/kg body weight; Santa Cruz Biotechnology, Cat# 37739-05-2) was administered intraperitoneally at -0.5, 1.5, and 3.5 h relative to the first kainic acid injection.

Analysis

EEG signals were high-pass filtered at 5 Hz and cleaned of line noise using a band-stop filter (48–50 Hz) and notch filters at 50, 100, and 150 Hz. Artifacts ("squid jumps") were identified as peaks exceeding 20 z-scores of the Teager–Kaiser Energy Operator (TKEO) on an unconnected channel. Epileptic spikes from the temporal electrode were defined as TKEO peaks exceeding 20 z-scores (relative to the 1-hour baseline) and a second derivative $>0.25 \text{ mV/s}^2$. Seizures were detected based on power spectral density in the 20–60 Hz range, calculated in 1-second bins using a sliding Hamming window. Power values were z-scored relative to the baseline hour. Events separated by $<10 \text{ s}$ were grouped as a single seizure; events $<5 \text{ s}$ were excluded.

Seizure behavior was scored blinded to experimental group using a modified Racine scale ⁶⁷: 0: no abnormal behaviors; 1: freezing; spikes without abnormal behavior; 2: myoclonic jerks, head nodding, tail stiffening; 3, forelimb clonus while sitting; 4, forelimb clonus with rearing and falling; 5, tonic-clonic convulsions with jumping, running and loss of righting reflex. One active-ASO-treated mouse reached a Racine score of 5 and was euthanized; missing values were imputed using the group mean. Seizure severity was quantified as the sum of seizure duration $\times$ Racine score across all time points for each mouse. Mice receiving ASO injections were sacrificed 7–10 days post-experiment to confirm successful TREM2 knockdown.

Brain-wide TREM2 knockdown by antisense oligomers

ASOs were obtained from Integrated DNA Technologies and stored at -20°C until use. The ASOs followed a gapmer design, consisting of five 2'-O-methoxyethyl-modified nucleotides at both the 5' and 3' ends. The active (5'-ATGGAGTGTATCCCCGACGC-3') and control ASO (5'-CCTATAGGACTATCCAGGAA-3') with no expected binding ⁶⁸ were ordered from Integrated DNA Technology. Stereotactic injections were performed as described in ⁶⁵. Mice were anesthetized and a small craniotomy was made above the lateral

ventricle (AP: −0.3 mm, ML: +1.0 mm). A blunt 33-gauge needle connected to a 5 µL Hamilton syringe was lowered to a depth of 2.5 mm and 100 µg of active or control ASO diluted in 5 µl HEPES-buffered aCSF (all in mM: 45 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, and 10 HEPES, pH 7.5, autoclaved and membrane-filtered) or vehicle was injected over 10 min. Mice recovered for at least 1 week before EEG implantation, and seizure experiments were performed 2–3 weeks after ASO injection.

AD patient-derived Aβ species extraction

Frozen cortical tissue was obtained from the Neuropathology Core of the Massachusetts ADRC (Massachusetts General Hospital), with all procedures approved under Partners Institutional Review Board protocol (Walsh BWH2011). Samples were collected from a 69-year-old female donor with end-stage AD, satisfying CERAD clinical criteria and Braak stage V pathological diagnosis. Amyloid plaques were isolated and Aβ purified as described previously^{26,69}. Aβ monomer and dimer preparations were stored at −80 °C and, directly prior to use, thawed and diluted to 50 ng/ml in aCSF.

Statistical analysis

All statistical analyses were performed in MATLAB using the following built-in functions: two-sided Wilcoxon signed-rank test (signrank), two-sided Wilcoxon rank-sum test (ranksum), one-way ANOVA followed by Tukey's post hoc test (anova1 and multcompare('CType','tukey-kramer')), Kruskal–Wallis test with Dunn–Sidak post hoc comparison (kruskalwallis and multcompare('CType','dunn-sidak')), and two-sample Kolmogorov–Smirnov test (kstest2) with custom-written Bonferroni correction. Results were considered significant at $p < 0.05$; p -values smaller than 0.001 are reported as $p < 0.001$. If not all p -values are shown in the figure, remaining tests were non-significant. Details on statistical tests, biological units, measures of center and dispersion, and precision are provided in the figure legends. Box plots indicate the median, interquartile range, and $1.5 \times \text{IQR}$, with individual observations overlaid.

Data availability

The TREM2^{H157Y} mouse line generated in this study has been deposited at the Jackson Laboratory (C57BL/6J-*Trem2*^{em3Bwef}/J, RRID: IMSR_JAX:033371). Source data and uncropped western blots

generated in this study is available in Figshare, MATLAB code to reproduce quantitative comparisons is available at Code Ocean.

Recombinant proteins are available from Michael Willem. Any additional information required to reanalyze the data reported in this paper is available from the lead contact, Benedikt Zott, upon request.

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Author information

Contributions

Conceptualization: FU, CW, KS, PP, AK, CH, MW, BZ; Data curation: FU, CW, KS, MW, BZ; Formal Analysis: FU, CW, KS, NU, PR, PP, LC, AKD, UG, FH, HM, AE, CG, BN, MW, BZ; Funding acquisition: AK, CH, BZ; Investigation: FU, CW, KS, NU, PR, PP, LC, BW, WW, AKD, WH, UG, HM, AE, CG, BN, BB, MW, BZ; Methodology: FU, KS, UG, FH, MW, BZ; Project administration: FU, BZ; Resources: BW, CG, AE, BN, IF, AK, CH, BZ; Software: FU, BZ; Supervision: DMW, AK, CH, MW, BZ; Validation: FU, CW, KW, MW;

650 Visualization: FU, CW, KS, NU, MW; Writing – original draft: FU, BZ; Writing – draft revision: FU, CW, KS,
651 MW, BZ; Writing – review & editing: all authors.

652 **Competing interests**

653 CH has a cooperation contract with Denali Therapeutics on TREM2 agonism. DMW is a former Biogen
654 employee and stockholder, consults for Acumen, Apellis, BrainQr, Convergen, Ecor1, Eisai, Etiome,
655 Kesmalea, Montara, the Dementia Discovery Fund, and Third Rock Ventures, and serves on the scientific
656 advisory board of Aerska, QUABY, and Tau Biosciences. All other authors declare no conflict of interest.

657 **Supplementary Information**

658 **Document S1. Sequences for expressed constructs and primers**

659

660

Fig 1

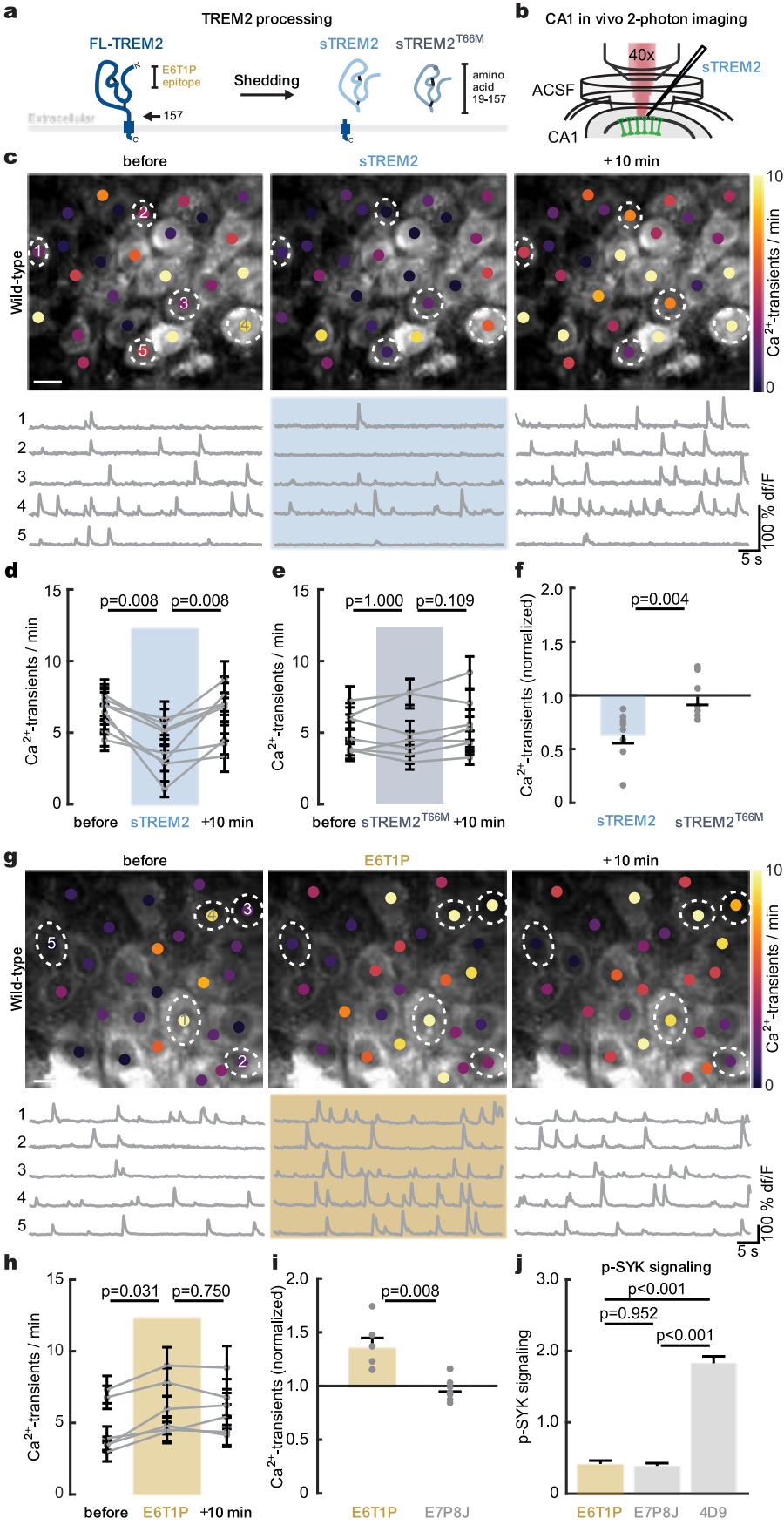


Figure 1. sTREM2 acutely dampens neuronal activity

(a) TREM2 processing at the microglial membrane. ADAM10/17-mediated cleavage at histidine 157 (arrow) releases soluble TREM2 (sTREM2, amino acids 19–157) from full-length TREM2 (FL-TREM2). T66M mutation (gray), E6T1P antibody epitope (orange).

(b) In vivo two-photon imaging setup for Ca^{2+} -imaging in CA1 hippocampal pyramidal cells. Pipette for sTREM2 application (blue). aCSF, artificial cerebrospinal fluid; CA1, cornu ammonis 1.

(c) (top) Two-photon images of CA1 neurons before, during, and 10 min after local application of sTREM2 (5 μM) in a wild-type mouse. Colored dots indicate Ca^{2+} transient frequency of individual neurons. (bottom) Traces from the five labeled neurons. sTREM2 application is indicated in blue.

(d) Quantification of the Ca^{2+} transient frequency before, during, and 10 min after sTREM2 application. Dots represent mouse means; gray lines connect repeated measurements ($n = 8$ mice).

(e) Quantification as in (d) for sTREM2^{T66M} application (5 μM ; $n = 6$ mice).

(f) Normalized activity during application of sTREM2 or sTREM2^{T66M} relative to baseline (sTREM2, $n = 8$ mice; sTREM2^{T66M}, $n = 6$ mice).

(g) As in (c) for local application of the anti-TREM2 antibody E6T1P (5 μM).

(h) Quantification as in (d) for E6T1P application (5 μM ; $n = 6$ mice).

(i) As in (f) for the application of E6T1P ($n = 6$ mice) or isotype control antibody E7P8J ($n = 8$ mice).

(j) AlphaLISA-based quantification of p-SYK signaling in HEK293 cells stably overexpressing mouse TREM2 and mouse DAP12 after 5 min treatment with E6T1P, E7P8J, or 4D9 antibody ($n = 3$ technical replicates from 2 independent experiments).

Scale bars in (c, g), 5 μm . Data presented as mean $\pm$ SEM. Wilcoxon signed-rank test (d, e, h). Wilcoxon rank sum test (f, i). One-way ANOVA ($p < 0.001$) followed by Tukey's post hoc test (j).

Fig 2

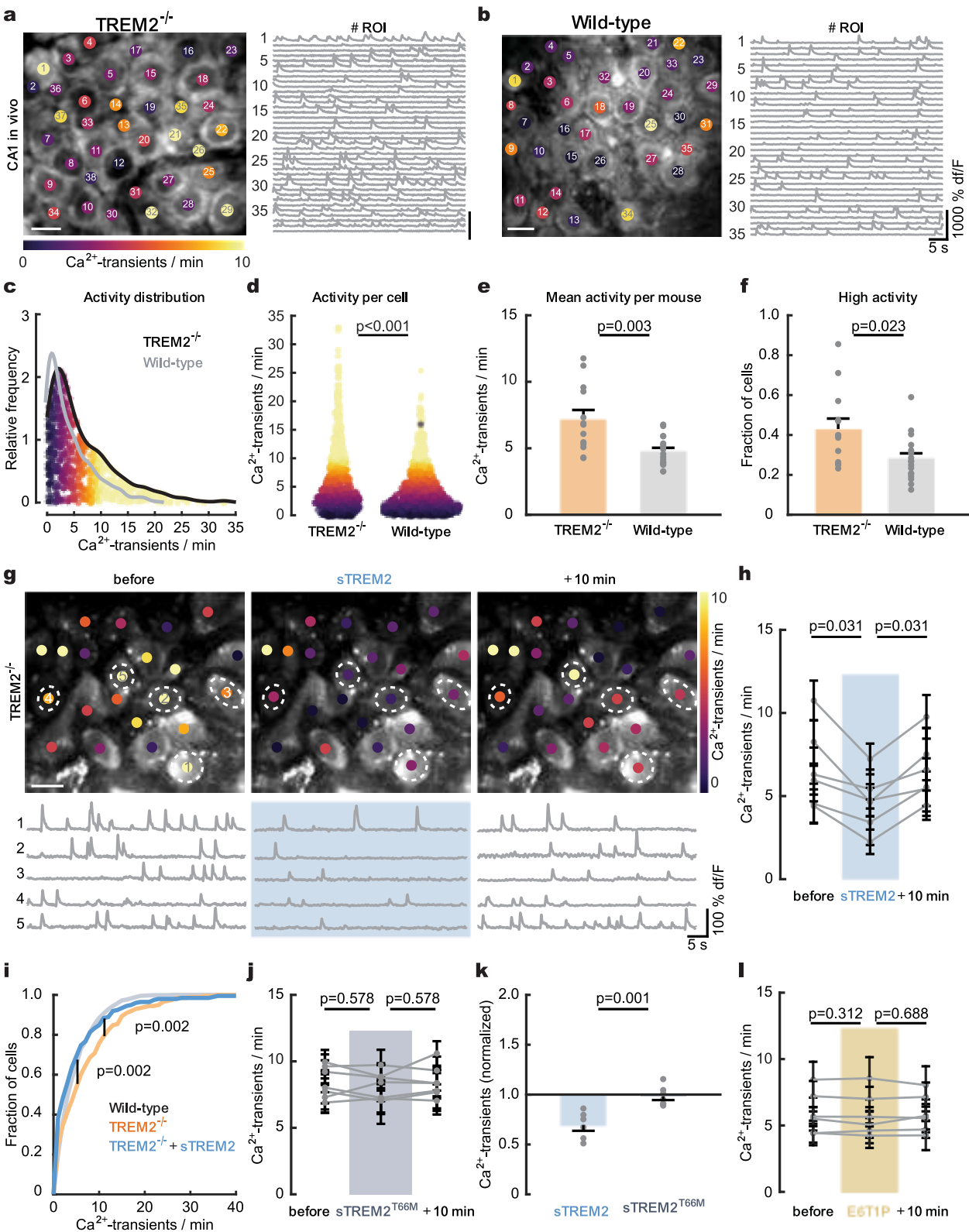


Figure 2. sTREM2 substitution reverses the neuronal hyperactivity phenotype of TREM2^{-/-} mice.

(a, b) Representative two-photon images (left) and Ca²⁺ traces (right) from a TREM2^{-/-} (a) and a wild-type (b) mouse. Colored dots indicate Ca²⁺ transient frequency; numbers indicate ROIs.

(c) Neuron-wise activity distribution in TREM2^{-/-} (n = 679 neurons from 13 mice) and wild-type (n = 805 neurons from 17 mice) animals. Each dot represents one neuron; the gray line marks the wild-type distribution.

(d) Ca²⁺ transient frequency of individual neurons in TREM2^{-/-} and wild-type mice. Each dot represents one neuron.

(e) Mean Ca²⁺ transient frequency per mouse in TREM2^{-/-} (n = 13 mice) and wild-type (n = 17).

(f) Fraction of highly active neurons (>6.4 Ca²⁺ transients/min) per mouse in TREM2^{-/-} and wild-type mice.

(g) Two-photon images of CA1 neurons before, during, and 10 min after local application of sTREM2 (5 μM) in a TREM2^{-/-} mouse. Colored dots indicate Ca²⁺ transient frequency; traces from the five labeled neurons are shown below.

(h) Quantification of the experiment in (g). Dots represent mouse means; gray lines connect repeated measurements (n = 6 mice).

(i) Cumulative distribution of neuronal activity in TREM2^{-/-} mice before (orange) and during (blue) sTREM2 application (n = 198 neurons from 6 mice), compared with untreated wild-type mice (n = 805 neurons from 17 mice).

(j) Quantification as in (h) for sTREM2^{T66M} application in TREM2^{-/-} mice (5 μM; n = 6 mice).

(k) Normalized activity during application of sTREM2 or sTREM2^{T66M} in TREM2^{-/-} mice (n = 6 mice, respectively).

(l) Quantification as in (h) for E6T1P application in TREM2^{-/-} mice (5 μM; n = 6 mice).

Scale bars: 5 μm. Data presented as mean ± SEM. Two-sample Kolmogorov-Smirnov test with Bonferroni correction (d, i). Wilcoxon rank sum test (e, f, k). Wilcoxon signed-rank test (h, j, l).

Fig 3

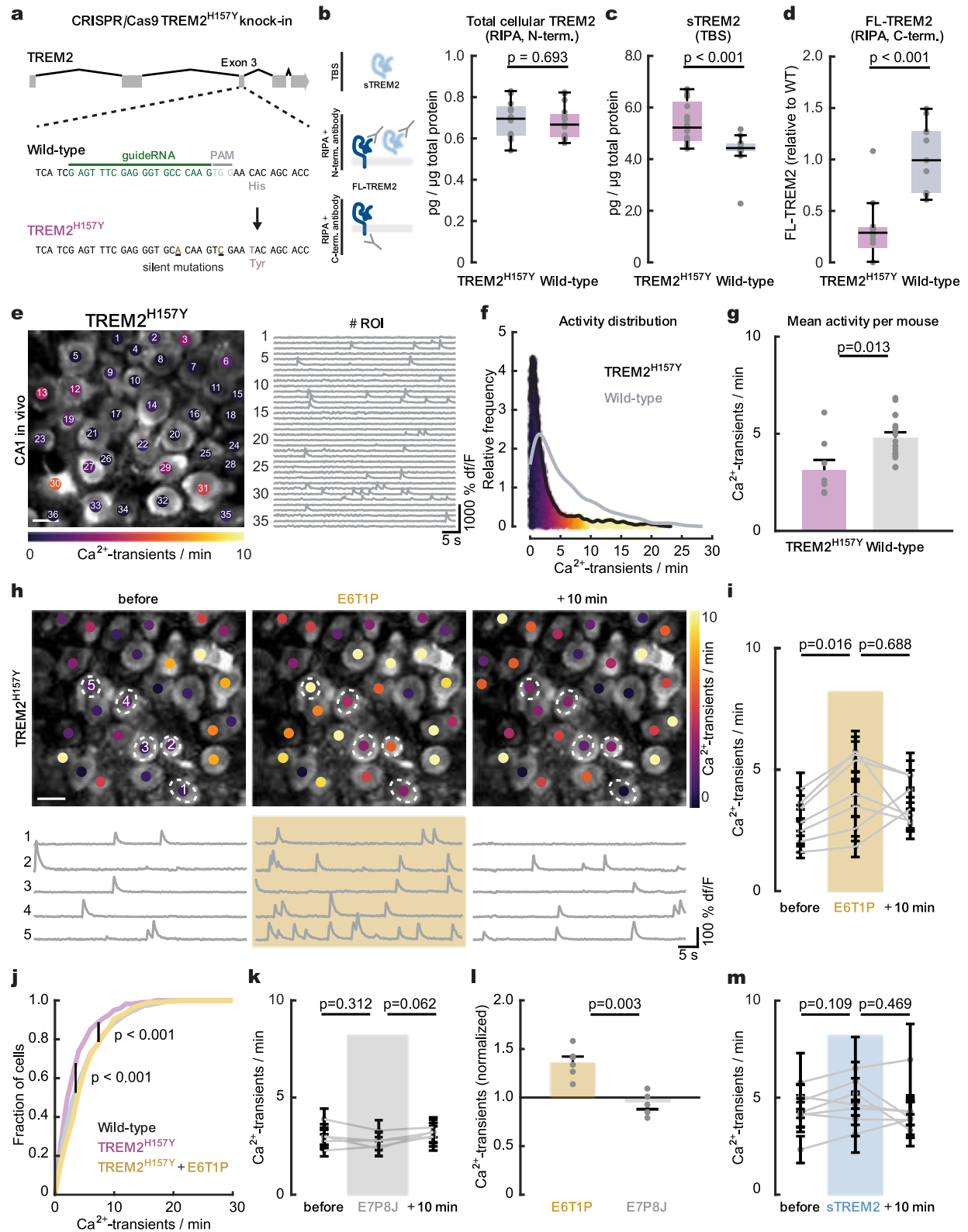


Figure 3. Increased sTREM2 levels are associated with lower neuronal activity in TREM2^{H157Y} mice.

(a) Generation of TREM2^{H157Y} knock-in mice by CRISPR/Cas9-mediated introduction of a C-to-T mutation in exon 3 (position 157, pink). Guide RNA target region (green), protospacer adjacent motif sequence (PAM, gray). Silent mutations introduced for genotyping are underlined.

(b) (left) Lysis-buffer-dependent enrichment of distinct TREM2 species. (right) ELISA-based quantification of total cellular TREM2, including FL-TREM2 and intracellular sTREM2, in RIPA brain lysates from 7-month-old TREM2^{H157Y} and wild-type mice (n = 16 and 12 mice).

(c) ELISA-based quantification of sTREM2 in TBS brain lysates from TREM2^{H157Y} and wild-type mice (n = 16 and 12 mice).

(d) Western blot-based quantification of FL-TREM2 in RIPA brain lysates using a C-terminal antibody (n = 13 and 11 mice), normalized to the wild-type mean.

(e) Two-photon image of CA1 pyramidal neurons and corresponding Ca²⁺ traces from a TREM2^{H157Y} mouse. Colored dots indicate Ca²⁺ transient frequency of individual neurons; numbers indicate ROIs.

(f) Neuron-wise activity distribution in TREM2^{H157Y} (n = 1085 neurons from 8 mice) compared with wild-type mice (n = 805 neurons from 17 mice). Each dot represents one neuron; the gray line marks the wild-type distribution.

(g) Mean Ca²⁺ transient frequency per mouse in TREM2^{H157Y} and wild-type mice. Each dot represents one mouse.

(h) Two-photon images of CA1 neurons before, during, and 10 min after local application of the anti-TREM2 antibody E6T1P (5 μM) in a TREM2^{H157Y} mouse. Traces from the five labeled neurons are shown below.

(i) Quantification of the experiment in (h). Dots represent mouse means; gray lines connect repeated measurements (n = 7 mice).

(j) Cumulative distribution of neuronal activity in TREM2^{H157Y} mice before and during E6T1P application (n = 222 neurons from 7 mice), compared to wild-type mice (n = 805 neurons from 17 mice).

737 (k) Quantification as in (i) for E7P8J application in TREM2^{H157Y} mice (5 μ M; n = 5 mice).

738 (l) Normalized activity during application of E6T1P (n = 7 mice) or E7P8J (n = 5 mice) antibodies relative
739 to baseline in TREM2^{H157Y} mice.

740 (m) Quantification as in (i) for sTREM2 application in TREM2^{H157Y} mice (5 μ M; n = 7 mice).

741 Scale bars in (e, h), 5 μ m. Data presented as mean $\pm$ SEM (g, i, k–m). Wilcoxon rank sum test (b–d, g, l).
742 Wilcoxon signed-rank test (i, k, m). Two-sample Kolmogorov-Smirnov test with Bonferroni correction (j).

743

Fig 4

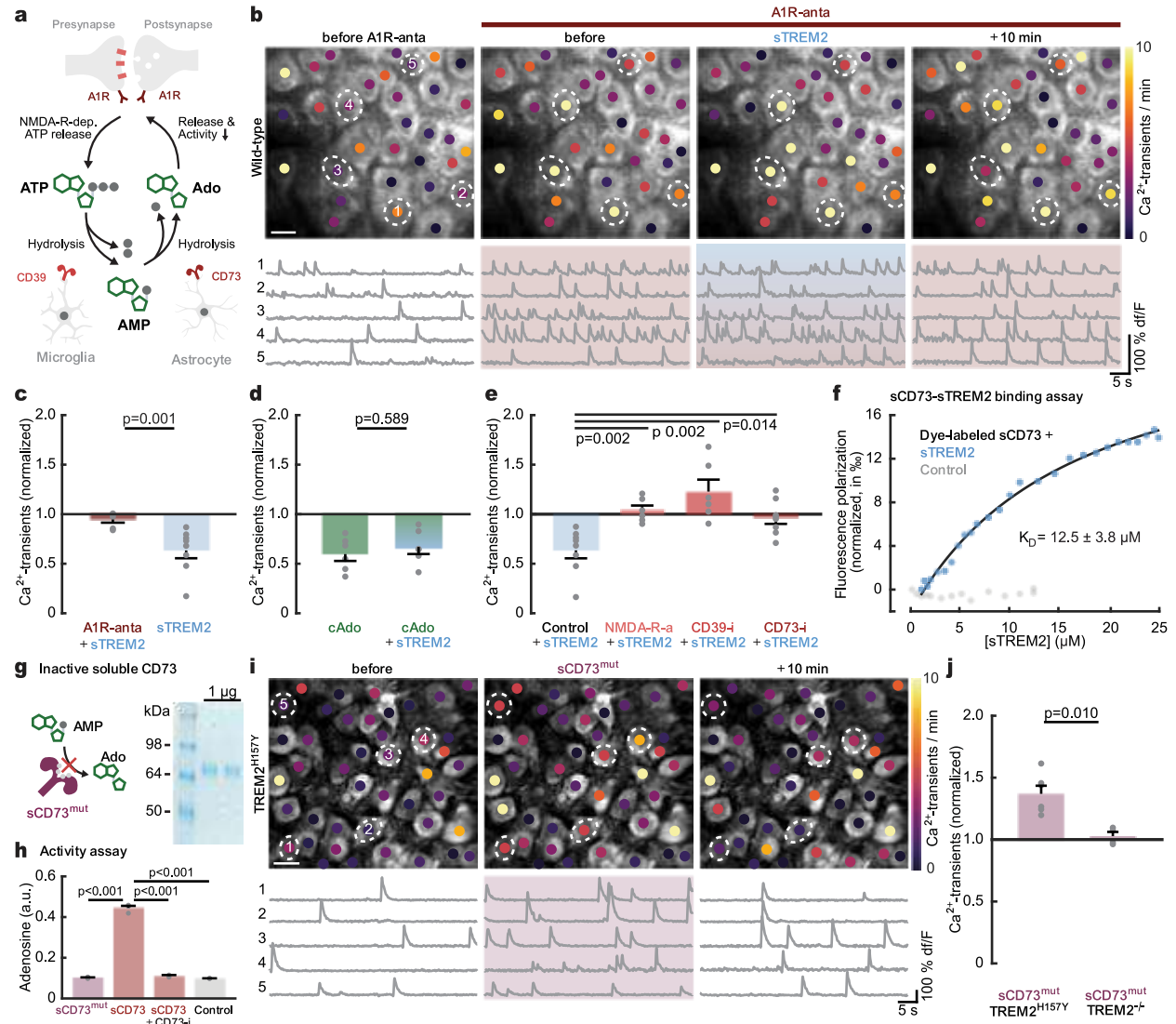


Figure 4. sTREM2 suppresses neuronal activity through CD73-dependent purinergic microglia–neuron signaling

(a) Schematic of purinergic microglia–neuron signaling.

(b) Two-photon images and Ca²⁺ traces before and during (30 min after onset) bath application of the A1R-antagonist DPCPX (A1R-anta, 1 μM), during additional local sTREM2 application (5 μM), and 10 min after sTREM2 washout in a wild-type mouse. Colored dots indicate Ca²⁺ transient frequency; traces from the five labeled neurons are shown below.

(c) Normalized activity during sTREM2 application in DPCPX-pretreated (n = 6) or non-pretreated wild-type mice (n = 8).

(d) Normalized activity during application of cAdo (0.125 μ M) or co-application of cAdo and sTREM2 (5 μ M) (n = 6 mice, respectively).

(e) Normalized activity during sTREM2 application in non-pretreated mice, or animals pretreated with the NMDA-R-antagonist APV (NMDA-R-a, 100 μ M), CD39-inhibitor ARL67156 (CD39-i, 100 μ M), or the CD73-inhibitor AOPCP (CD73-i, 100 μ M) (n = 8, 6, 6 and 6 mice, respectively).

(f) Fluorescence polarization assay using fluorescein-labeled soluble CD73 (sCD73) or free dye upon stepwise addition of sTREM2. KD values were derived by fitting to a one-site binding model using the Morrison equation (n = 8 independent experiments).

(g) (left) Schematic of a putative decoy receptor effect of recombinant catalytically inactive sCD73^{mut}. (right) Coomassie-stained SDS-PAGE of sCD73^{mut}. The full gel is provided in Extended Data Fig. 9e.

(h) Colorimetric adenosine production assay from AMP in the presence of sCD73^{mut}, sCD73, sCD73 + CD73- AOPCP (100 μ M), or no-enzyme control (n = 4, 4, 2, and 2 technical replicates from 2 experiments).

(i) Two-photon images of CA1 neurons before, during, and 10 min after local application of sCD73^{mut} (10 μ M) in a TREM2^{H157Y} mouse. Colored dots indicate Ca²⁺ transient frequency; traces from the five labeled neurons are shown below.

(j) Normalized activity during sCD73^{mut} application in TREM2^{H157Y} and TREM2^{-/-} mice relative to baseline (TREM2^{H157Y}, n = 6 mice; TREM2^{-/-}, n = 4 mice).

Scale bars in (b, i), 5 μ m. Data presented as mean $\pm$ SEM (c-e, h, j). Wilcoxon rank sum test (c, d, e, j) One-way ANOVA (p = 0.010) followed by Tukey's post hoc test (h).

Fig 5

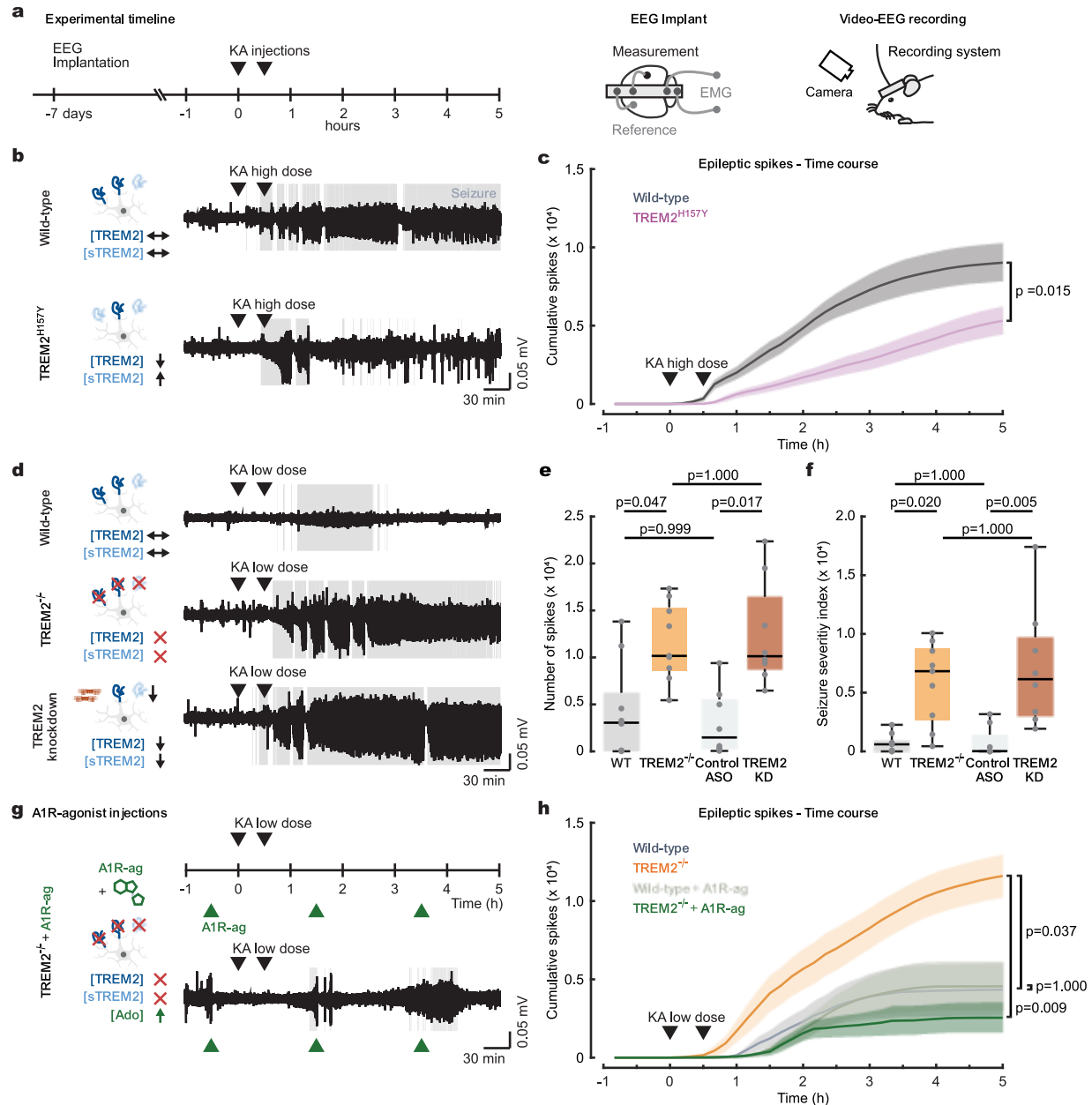


Figure 5. sTREM2 protects against seizures via A1R agonism in the KA seizure model

(a) Experimental timeline, EEG implant configuration, and video-EEG recording setup. Arrowheads indicate subcutaneous kainic acid (KA) injections at 0 and 0.5 h. EMG, electromyography.

(b) Representative EEG recordings from a wild-type and a $TREM2^{H157Y}$ mouse after two high-dose KA injections (10 mg/kg body weight). Detected seizures are indicated as grey boxes.

781 (c) Cumulative epileptic spike counts in TREM2^{H157Y} (n = 9 mice) and wild-type (n = 8) mice after high-
782 dose KA injections.

783 (d) Representative EEG recordings from a wild-type, a TREM2^{-/-}, and a TREM2 knockdown mouse after
784 two low-dose KA injections (5 mg/kg body weight).

785 (e) Number of epileptic spikes during the 5 h recording period in wild-type, TREM2^{-/-}, control-ASO or
786 vehicle-treated, and TREM2 knockdown mice (n = 9, 9, 8, and 8 mice, respectively).

787 (f) Seizure severity index, calculated as the time-weighted sum of Racine scores, for the experiment
788 shown in (e).

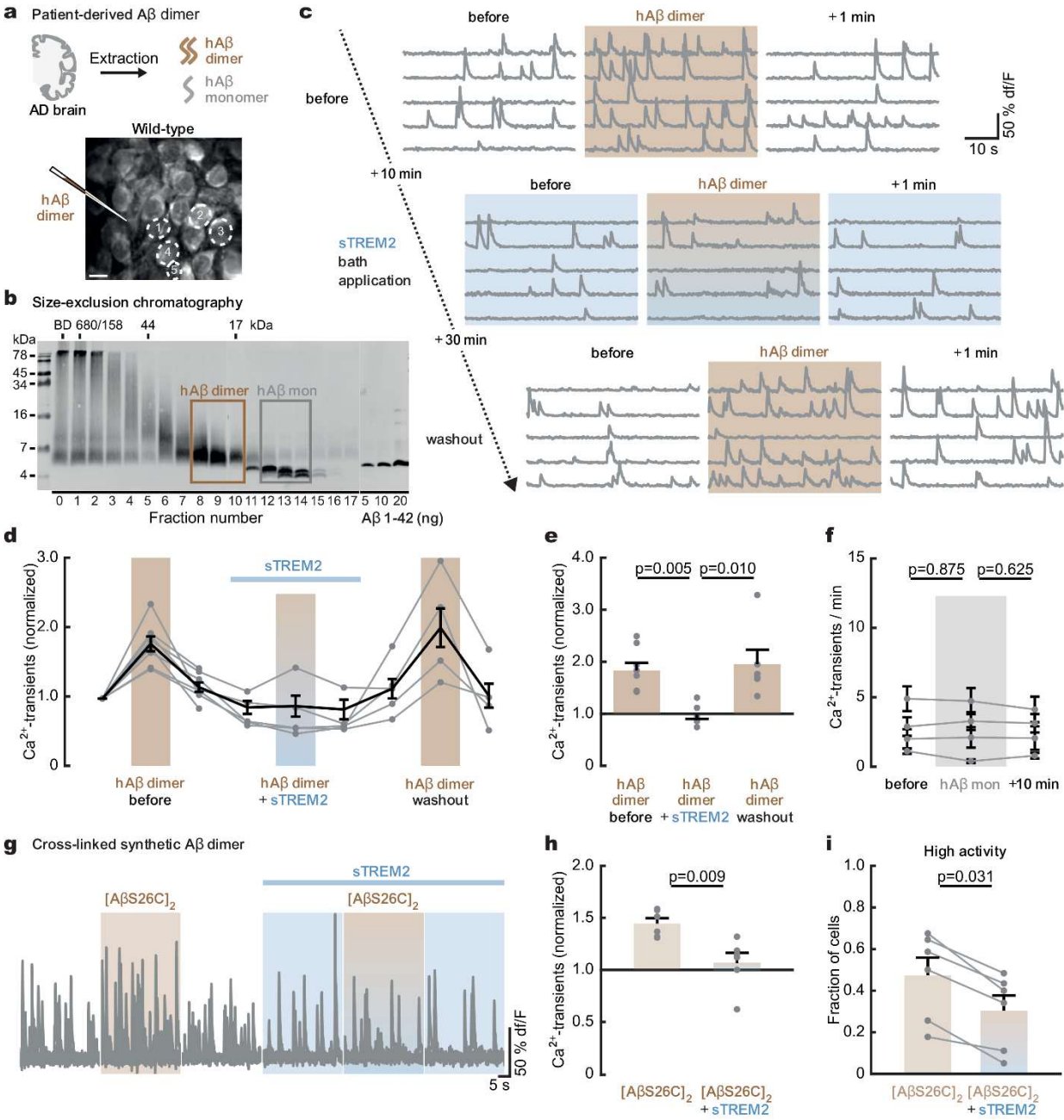
789 (g) Experimental timeline with additional intraperitoneal injections of the A1R agonist CCPA (A1R-ag; 3
790 mg/kg body weight) at -0.5, 1.5, and 3.5 h, and representative EEG recording from a TREM2^{-/-} mouse
791 receiving low-dose KA and A1R-ag.

792 (h) Cumulative epileptic spike counts in wild-type and TREM2^{-/-} mice after low-dose KA injections with or
793 without A1R-ag treatment. Thick lines indicate group means; shaded areas indicate SEM (without A1R-
794 ag, n = 9 mice each; with A1R-ag, n = 6 mice each).

795 Data presented as mean ± SEM (c, h) or boxplots (e, f). Wilcoxon rank sum test (c). Kruskal-Wallis test
796 with Dunn-Sidak post hoc comparison (e, f, h).

797

Fig 6



798

799

Figure 6. sTREM2 prevents human A β dimer–induced neuronal hyperactivation

(a) A β extraction from an AD patient. Representative two-photon image of hippocampal CA1 in a wild-type mouse with the A β application pipette.

(b) Western blot of size exclusion chromatography-fractionated human A β from formic acid-solubilized plaques using the 4G8 antibody. Fractions containing A β dimers (hA β dimer, fractions 8–10) and monomers (hA β mon, fractions 12–14) are boxed. The elution of blue dextran (BD) and globular protein standards is indicated on the top. A β _{1–42} was loaded for reference.

(c) Ca²⁺ traces from the five labeled neurons in (a) before, during, and 1 min after hA β dimer application (50 ng/ml), followed by the same sequence during sTREM2 bath application (10 nM) and after 30 min sTREM2 washout.

(d) Quantification of the experiment shown in (c). Gray lines connect repeated measurements from the same mouse; activity was normalized to the first recording (n = 8 mice initially).

(e) Normalized hA β dimer effect shown in (d) under control, sTREM2, and washout conditions. Values were normalized to the respective baseline activity (n = 8, 6, and 6 mice).

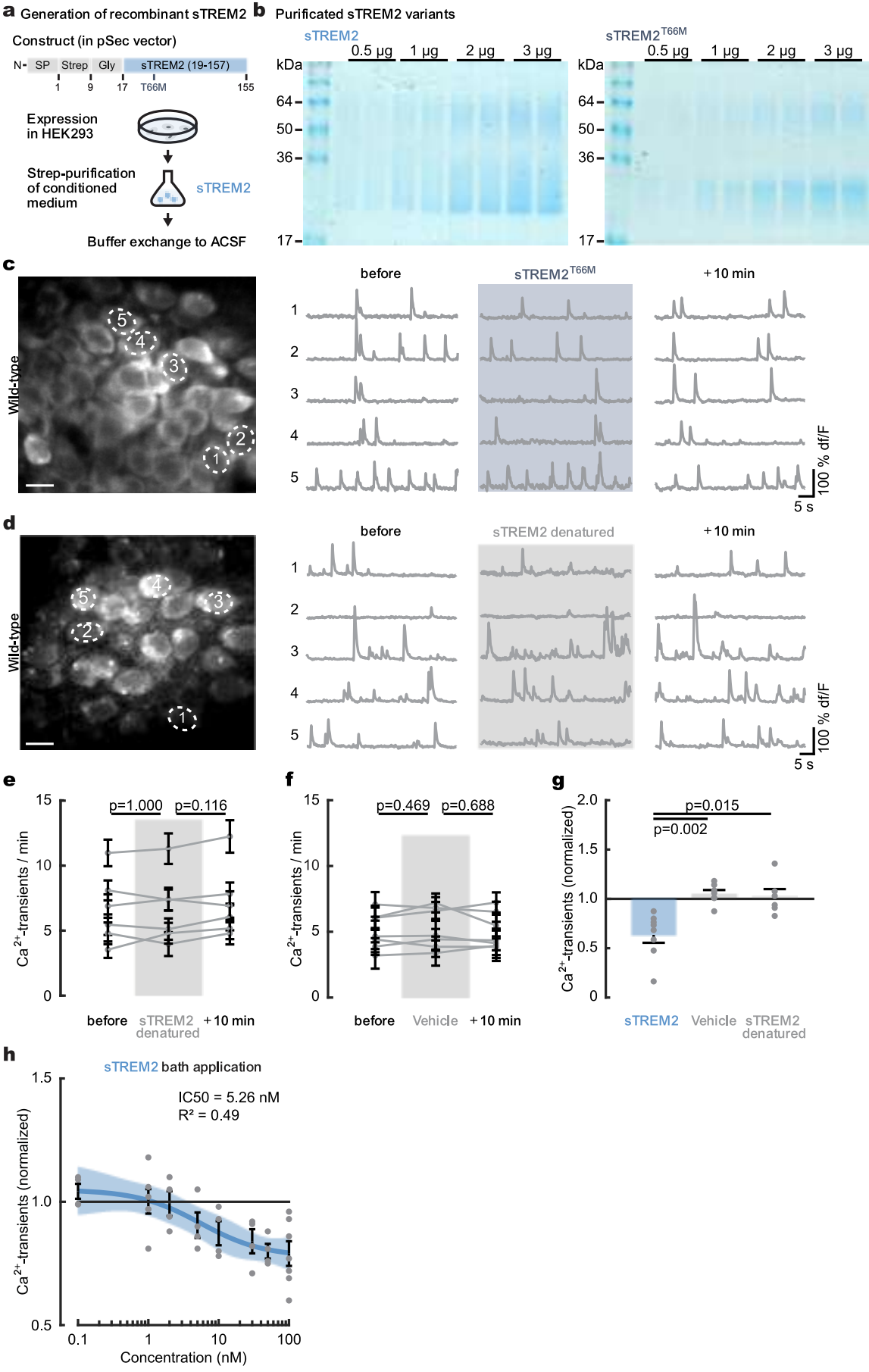
(f) Quantification of Ca²⁺ transient frequency before, during, and 1 min after application of human A β monomers (hA β mon; 50 ng/ml, n = 4 mice).

(g) Superimposed Ca²⁺ traces from representative neurons before, during, and 1 min after [A β S26C]₂ application (1 μ M), followed by the same sequence during bath application of sTREM2 (10 nM).

(h) Normalized effect of [A β S26C]₂ application under control conditions and during sTREM2 bath application (n = 6 mice, respectively).

(i) Fraction of highly active neurons during [A β S26C]₂ application under control conditions and during sTREM2 bath application (n = 6 mice).

Scale bar in (a), 5 μ m. Data presented as mean $\pm$ SEM. Kruskal-Wallis test (e). Wilcoxon signed-rank test (f, i). Wilcoxon rank sum test (h).



Extended Data Figure 1. sTREM2 and sTREM2^{T66M} variant generation and control experiments

(a) Schematic of recombinant sTREM2 generation in HEK293 cells. The construct contains an N-terminal Strep-tag II, an 8-amino-acid glycine linker, and murine sTREM2 (amino acids 19–157) in a pSec vector containing a signal peptide (SP). Conditioned medium was collected, Strep-tag affinity-purified, and buffer-exchanged into aCSF. See Document S1 for full constructs.

(b) Coomassie-stained SDS-PAGE of purified recombinant sTREM2 and sTREM2^{T66M} loaded at increasing amounts per lane with monomers at ~30 kDa and dimers at ~60 kDa.

(c) Representative two-photon image and Ca²⁺ traces before, during, and 10 min after local application of recombinant sTREM2^{T66M} (5 μM) in a wild-type mouse.

(d) Representative two-photon image and Ca²⁺ traces before, during, and 10 min after local application of heat-denatured sTREM2 (5 μM) in a wild-type mouse.

(e) Quantification of Ca²⁺ transient frequency before, during, and 10 min after heat-denatured sTREM2 application. Each dot represents the mean activity of all imaged neurons from one mouse; gray lines connect repeated measurements from the same mouse (n = 6 mice).

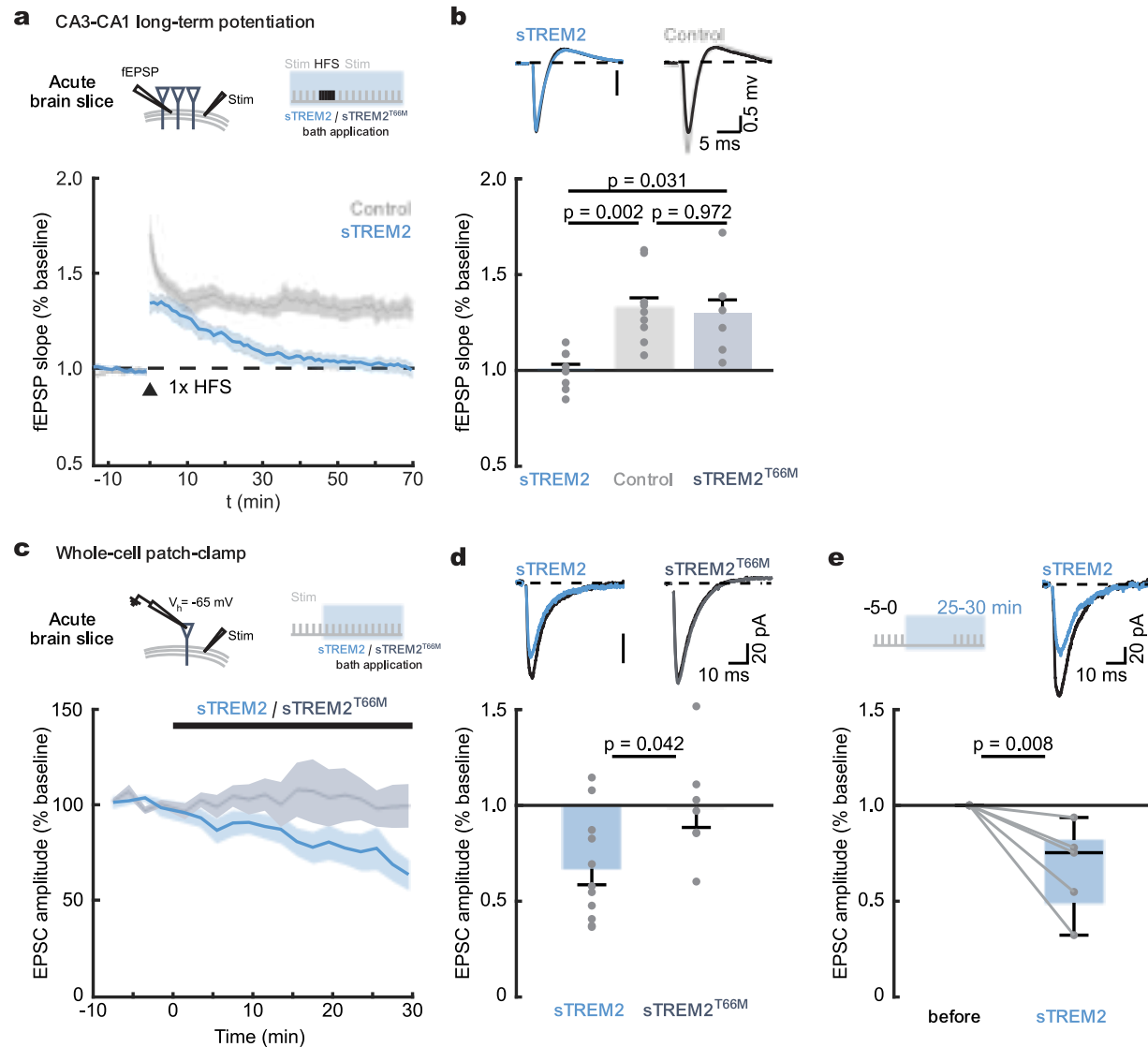
(f) Quantification as in (e) for vehicle application in wild-type mice (pooled for aCSF and PBS, n = 4 mice with aCSF, n = 3 with PBS).

(g) Normalized activity during the application of sTREM2, vehicle, or heat-denatured sTREM2 relative to baseline (n = 8, 7 and 6 mice).

(h) Dose-response curve for bath-applied sTREM2 in wild-type mice. Activity was measured 10–20 min after sTREM2 addition to circulating aCSF and normalized to pre-application activity. The line indicates a four-parameter logistic fit; shaded area indicates the 95% confidence interval.

Scale bars in (c, d), 5 μm. Data presented as mean ± SEM. Wilcoxon signed-rank test (e, f). Kruskal-Wallis test with Dunn-Sidak post-hoc comparison (g). Four-parameter logistic fit in (h).

Extended Data 2



850

851 **Extended Data Figure 2. sTREM2 suppresses LTP and decreases EPSC amplitude.**

852 (a) (top left) Placement of the stimulation (Stim) and recording pipette (fEPSP). (top right) stimulation
 853 paradigm. (bottom) Baseline-normalized fEPSP slope under Schaffer collateral stimulation in acute brain
 854 slices from 4–8-week-old wild-type mice under control conditions or sTREM2 bath application (50 nM).
 855 Arrow indicates LTP induction. Data presented as mean $\pm$ SEM (shaded area; sTREM2, $n = 7$; control, n
 856 $= 9$ slices). fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation (1 burst, 100
 857 pulses at 100 Hz); Stim, continuous stimulation (0.05 Hz).

858 (b) (top) Averaged fEPSP traces before (black) and after LTP induction (colored) under control and
859 sTREM2 application conditions. (bottom) Baseline-normalized fEPSP slope 60–70 min after LTP
860 induction for slices incubated with sTREM2 (50 nM, n = 7), vehicle (n = 9), or sTREM2^{T66M} (50 nM, n = 8).

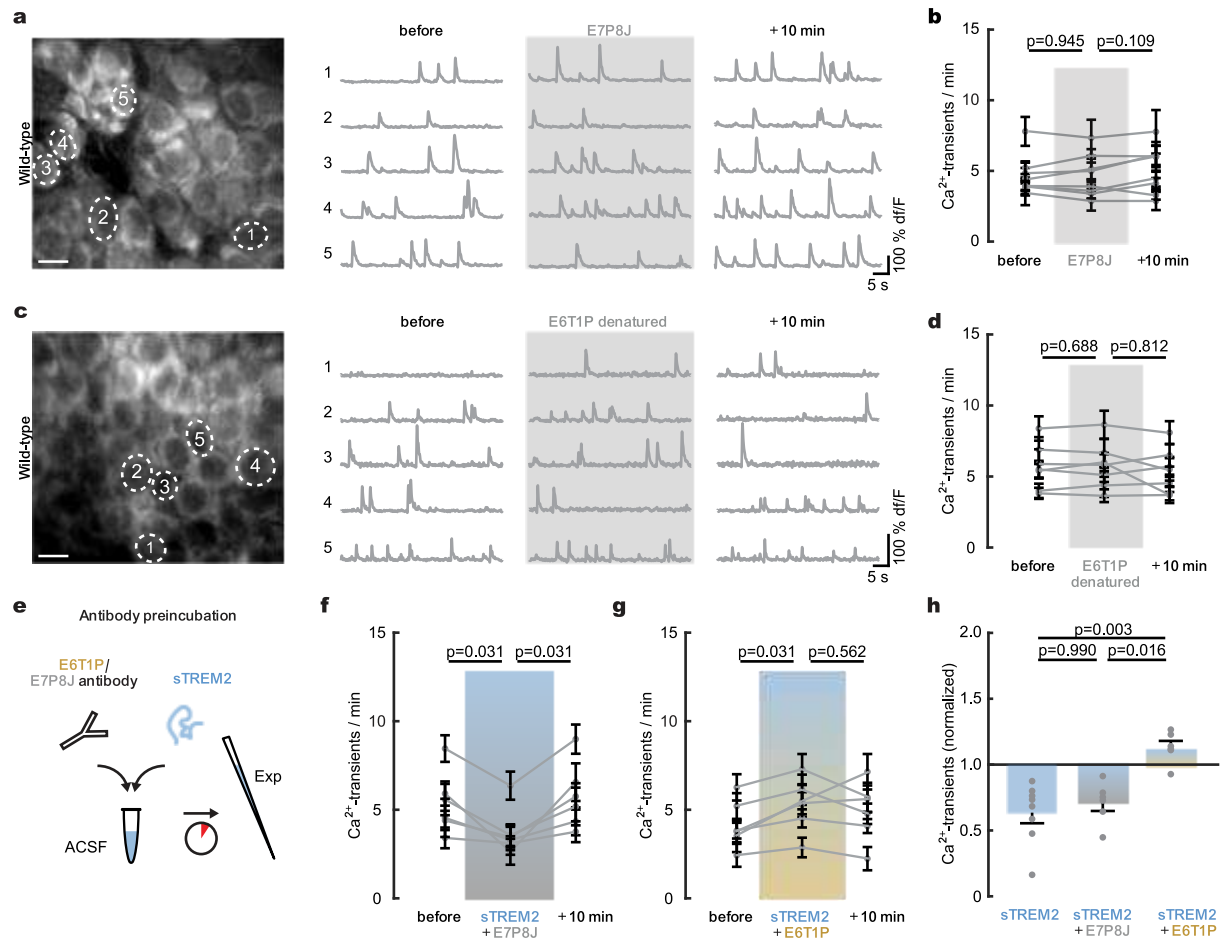
861 (c) (top left) Placement of stimulation (Stim) and patch pipettes. (top right) stimulation paradigm
862 (continuous stimulation, 0.0667 Hz). (bottom), baseline-normalized EPSC amplitude under Schaffer
863 collateral stimulation in voltage-clamped CA1 pyramidal neurons from wild-type brain slices during bath
864 application of sTREM2 or sTREM2^{T66M} (50 nM). Data presented as mean ± SEM (shaded area; n = 10
865 and 6 cells, respectively).

866 (d) (top) Averaged EPSCs before (black) and during sTREM2 or sTREM2^{T66M} application (colored).
867 (bottom) Normalized EPSC amplitudes after 25–30 min application. Each dot represents one neuron.

868 (e) (top left) Stimulation paradigm with stimulation paused during sTREM2 application (shaded area); (top
869 right) averaged EPSCs before (black) and after sTREM2 (blue). (bottom) normalized EPSC amplitude
870 before and after sTREM2 application (50 nM; n = 5 cells).

871 Data presented as mean ± SEM (b, d, e). Kruskal–Wallis test with Dunn–Sidak post hoc comparison (b);
872 Wilcoxon rank-sum test (d); Paired-sample t-test (e)

873



874

875 **Extended Data Figure 3. Controls for anti-TREM2 antibody experiments**

876 (a) Representative two-photon image and Ca^{2+} traces from the five labeled neurons before, during, and
 877 10 min after acute application of the E7P8J antibody (5 μM) in a wild-type mouse.

878 (b) Quantification of the experiment in (a). Each dot represents mean Ca^{2+} transient frequency of all
 879 imaged neurons from one mouse, before, during (E7P8J), and 10 min after application ($n = 8$ mice).

880 (c) As in (a), for heat-denatured E6T1P (5 μM).

881 (d) Quantification of the experiment in (c) ($n = 7$ mice). Each dot represents mean Ca^{2+} transient
 882 frequency of all imaged neurons from one mouse, before, during (heat denatured E6T1P), and 10 min
 883 after application ($n = 7$ mice).

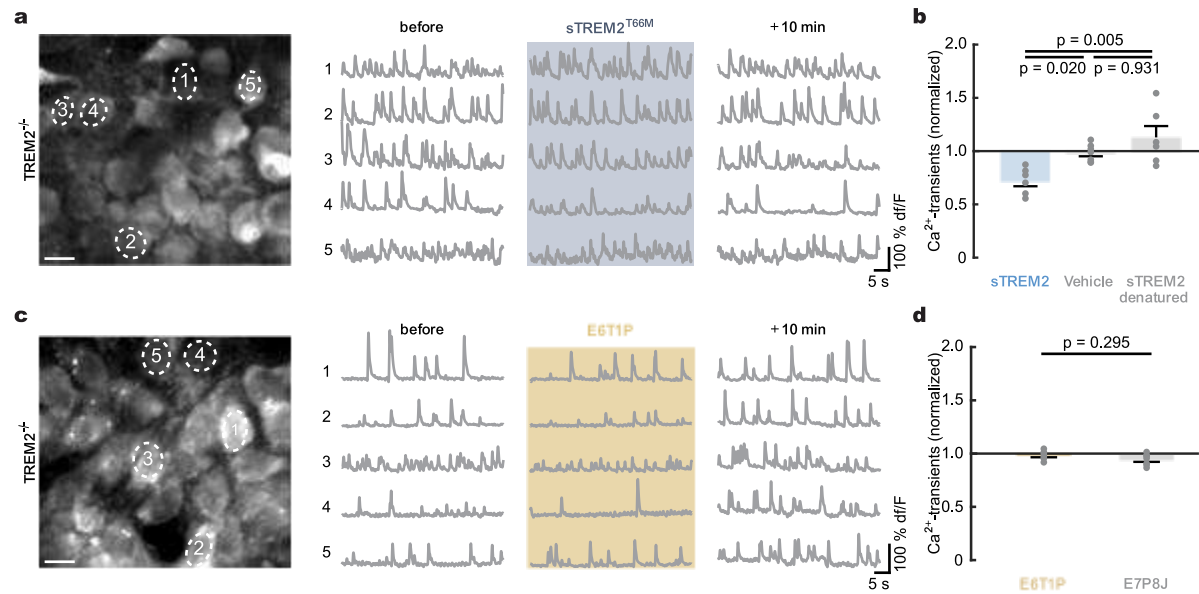
884 (e) Schematic of antibody preincubation: E6T1P or E7P8J was preincubated with sTREM2 (equimolar) for
885 1 h before co-application.

886 (f and g) Ca^{2+} transient frequency before, during, and 10 min after application of sTREM2 preincubated
887 with E7P8J (f) or E6T1P (g) (5 μM each).

888 (h) Normalized activity during application of sTREM2 alone (left, n = 8 mice), sTREM2 + E7P8J (middle, n
889 = 6), or sTREM2 + E6T1P (right, n = 6).

890 Scale bars in (a, c), 5 μm . Data presented as mean $\pm$ SEM. Two-sided Wilcoxon signed-rank test (b, d, f,
891 g). Kruskal-Wallis test with Dunn-Sidak post hoc comparison (h).

892



Extended Data Figure 4. Control experiments in TREM2^{-/-} mice.

(a) Representative two-photon image and Ca²⁺ traces from the five labeled neurons before, during, and 10 min after acute application of sTREM2^{T66M} (5 μ M) in TREM2^{-/-} mice. Quantification shown in Fig. 2j.

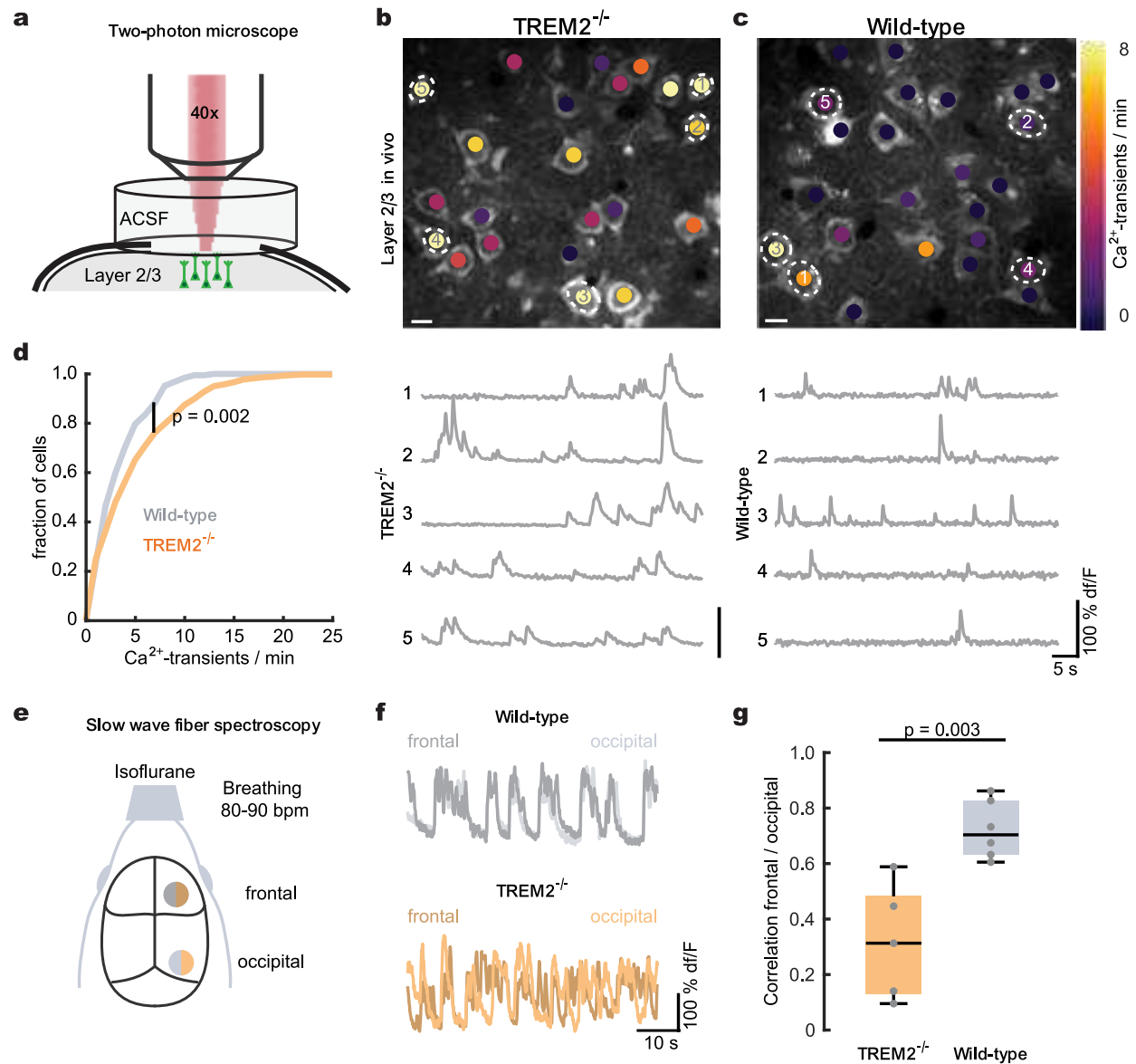
(b) Ca²⁺ transient frequency during application of sTREM2 (5 μ M), vehicle (aCSF), or heat-denatured sTREM2 (5 μ M) in TREM2^{-/-} mice, normalized to each mouse's baseline (n = 6, 7, and 6 mice, respectively).

(c) As in (a), for E6T1P (5 μ M) in TREM2^{-/-} mice. Quantification shown in Fig. 2l.

(d) As in (b), for E6T1P or E7P8J (5 μ M) in TREM2^{-/-} mice (n = 6 and 7 mice, respectively).

Scale bars in (a, c), 5 μ m. Data presented as mean $\pm$ SEM. Kruskal-Wallis test with Dunn-Sidak post hoc comparison (b). Wilcoxon rank sum test (d).

Extended Data 5



Extended Data Figure 5. Aberrant cortical neuronal activity in $TREM2^{-/-}$ mice

(a) Schematic of the two-photon imaging setup for in vivo Ca^{2+} -imaging of layer 2/3 pyramidal neurons in frontal cortex. aCSF, artificial cerebrospinal fluid.

(b and c) Representative two-photon images of Cal-520-AM-stained pyramidal neurons in the cortex of a 4–6-month-old $TREM2^{-/-}$ (b) and a wild-type (c) mouse. Dot color indicates Ca^{2+} transient frequency.

Traces from the five labeled neurons are shown below.

912 (d) Cumulative distribution of activity of individual cortical neurons in TREM2^{-/-} and wild-type mice
913 (TREM2^{-/-} n = 342 neurons from 5 mice; wild-type, n = 445 neurons from 3 mice).

914 (e) Schematic of fiber fluorometry of Ca²⁺ slow waves in 2–3-month-old wild-type or TREM2^{-/-} mice after
915 OGB-1 staining under light anesthesia.

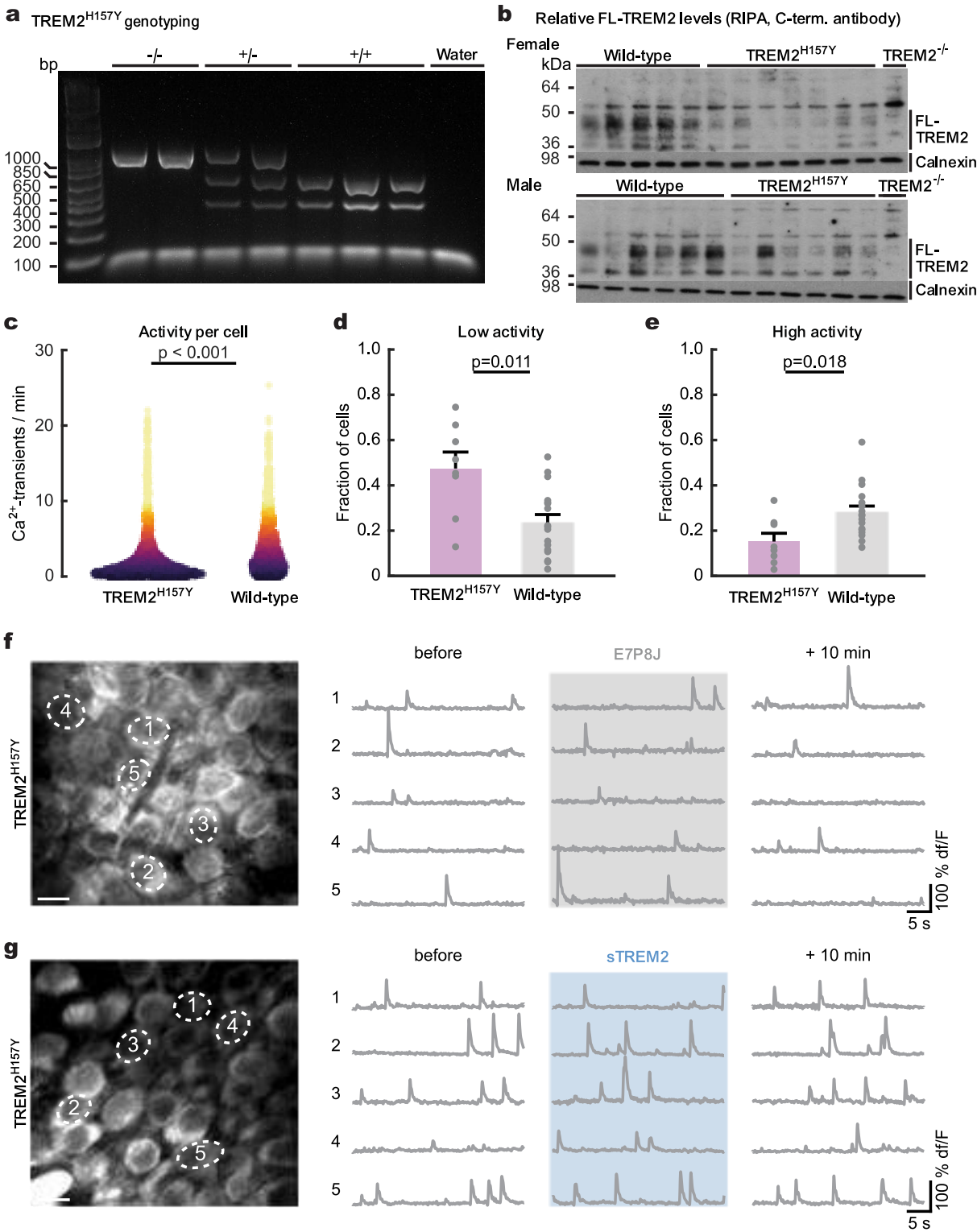
916 (f) Representative slow-wave traces from frontal (darker) and occipital (lighter) cortex in a wild-type (top)
917 and TREM2^{-/-} (bottom) mouse.

918 (g) Frontal–occipital Pearson correlation in TREM2^{-/-} and wild-type mice (n = 5 and 6 mice, respectively).

919 Scale bars in (b, c), 5 μm. Two-sample Kolmogorov-Smirnov test (d). Wilcoxon rank sum test (g).

920

Extended Data 6



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Extended Data Figure 6. Characterization of the TREM2^{H157Y} knock-in mice and control experiments

(a) Genotyping by PCR and Alw44I restriction digest. Homozygous (-/-) shows the undigested band, homozygous (+/+) shows the digested fragments, heterozygous (+/-) shows both. DNA ladder and water control are indicated.

(b) Western blots of RIPA brain lysates from 7-month-old wild-type and TREM2^{H157Y} mice, females (n = 5 and 7, respectively) and males (n = 6 each), probed with an antibody against the C-terminal sequence present only in FL-TREM2. One TREM2^{-/-} mouse was included to assess background, enabling accurate quantification of FL-TREM2 levels. Calnexin served as loading control. Quantification shown in Fig. 3d.

(c) Swarm plot of neuronal activity per cell in TREM2^{H157Y} and wild-type mice; each dot represents one neuron (TREM2^{H157Y}, n = 1085 neurons from 8 mice; wild-type, n = 805 neurons from 17 mice).

(d) Fraction of low-activity neurons (<1.2 Ca²⁺ transients/min) per mouse in TREM2^{H157Y} and wild-type mice (n = 8 and 17 mice).

(e) Fraction of high-activity neurons (>6.4 Ca²⁺ transients/min) per mouse in TREM2^{H157Y} and wild-type mice (n = 8 and 17 mice).

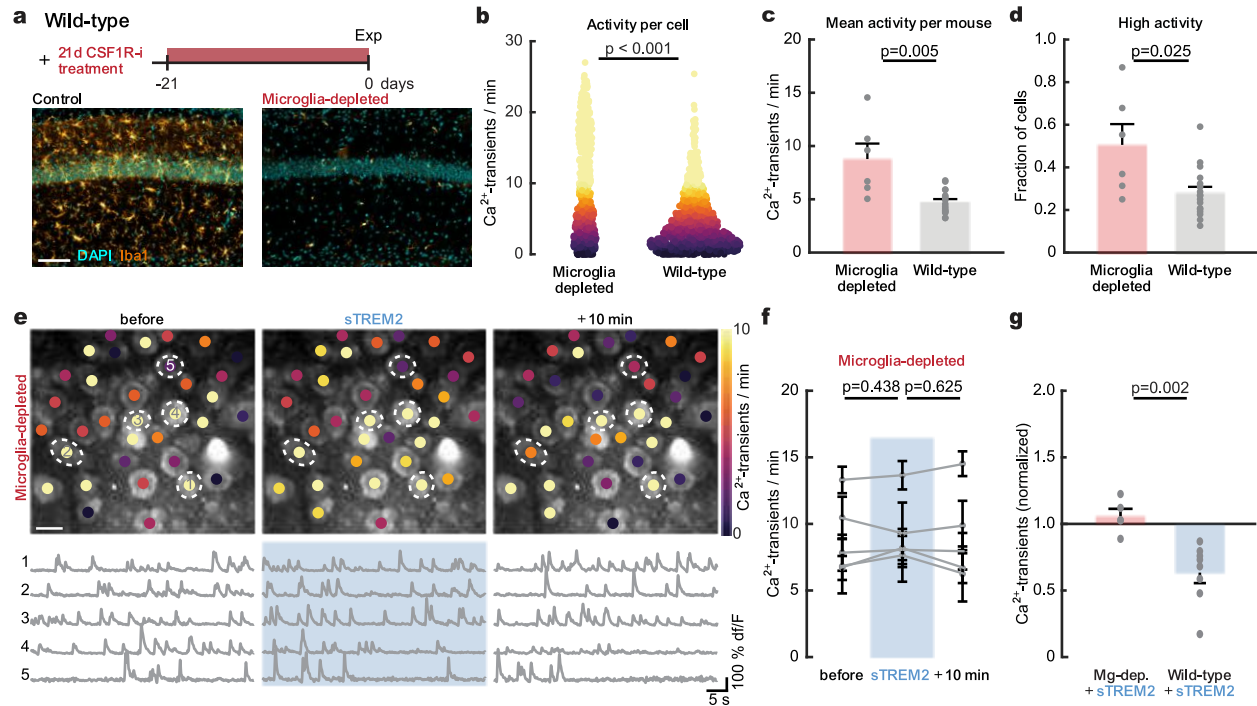
(f) Representative two-photon image and Ca²⁺ traces from the five labeled neurons before, during, and 10 min after acute application of E7P8J (5 μM) in TREM2^{H157Y} mice. Quantification shown in Fig. 3k.

(g) As in (f), for sTREM2 (5 μM) in TREM2^{H157Y} mice. Quantification shown in Fig. 3m.

Scale bars in (f, g), 5 μm. Data presented as mean ± SEM (d, e). Two-sample Kolmogorov-Smirnov test

(c). Wilcoxon rank sum test (d, e).

Extended Data 7



Extended Data Figure 7. Microglia depletion induces neuronal hyperactivity and abolishes sTREM2-mediated suppression of neuronal activity

(a) Schematic of microglia depletion in 3–5-month-old wild-type mice using chow containing the CSF1R inhibitor PLX5622 (CSF1R-i, 1200 mg/kg). Exp, day of experiment. Confocal images of CA1 from control and microglia-depleted mice are shown below. Iba1, orange; DAPI, cyan.

(b) Swarm plot of neuronal activity per cell in microglia-depleted (PLX5622 chow, CSF1R-i, 1200 mg/kg) and control (standard chow) wild-type mice; each dot represents one neuron (microglia-depleted, $n = 517$ neurons from 6 mice; wild-type, $n = 805$ neurons from 17 mice).

(c) Mean Ca²⁺ transient frequency per mouse in microglia-depleted and wild-type mice; each dot represents one mouse ($n = 6$ and 17 mice).

(d) Fraction of high-activity neurons (>6.4 Ca²⁺ transients/min) per mouse in microglia-depleted and wild-type mice ($n = 6$ and 17 mice).

956 (e) Two-photon images of CA1 neurons before, during, and 10 min after local application of sTREM2 (5
957 μM) in a microglia-depleted mouse. Colored dots indicate Ca^{2+} transient frequency; traces from the five
958 labeled neurons are shown below.

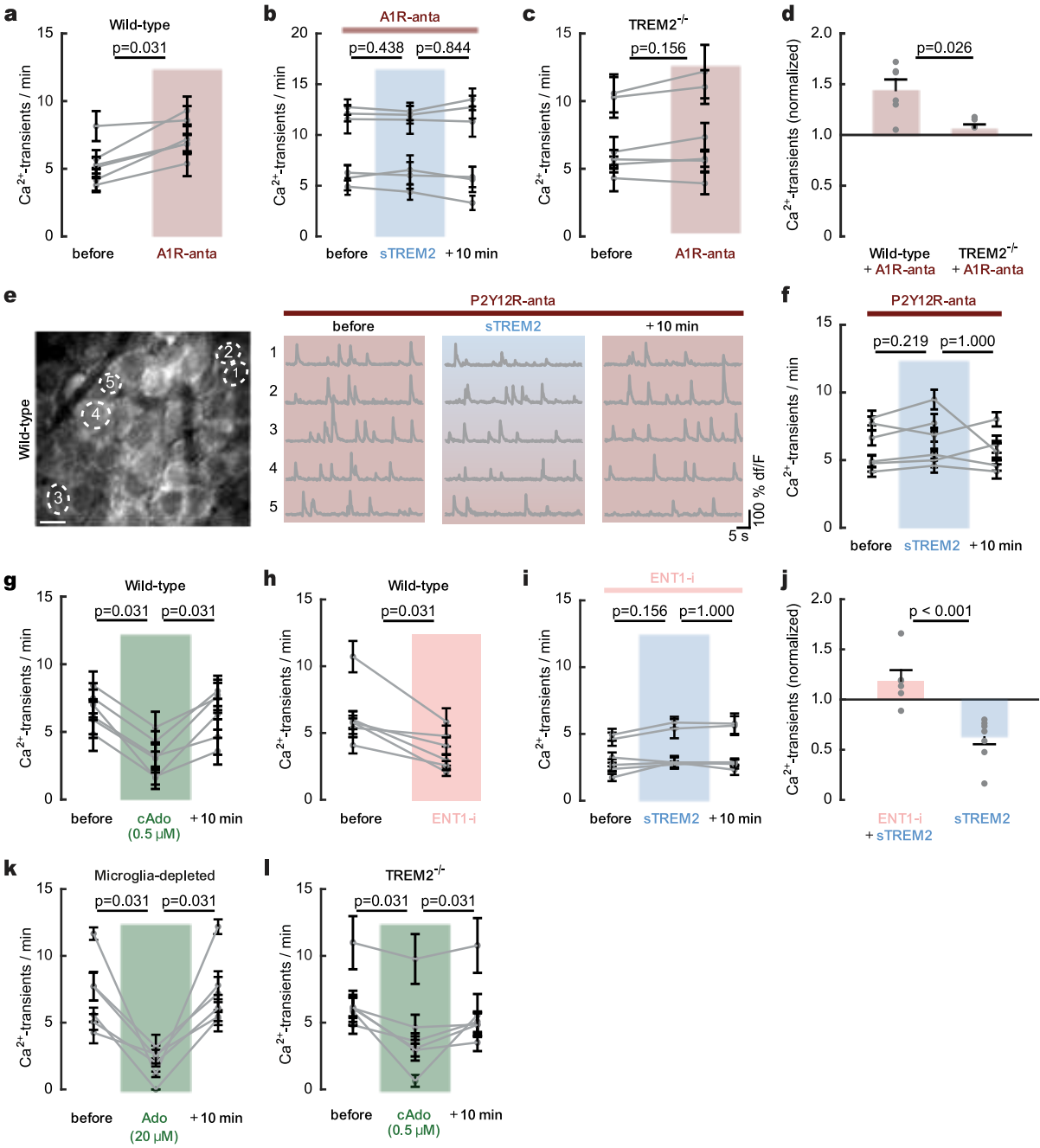
959 (f) Quantification of the experiment in (e). Each dot represents the mean activity of all imaged neurons
960 from one mouse. Gray lines connect repeated measurements from the same mouse ($n = 5$ mice).

961 (g) Normalized activity during sTREM2 application (5 μM) in microglia-depleted and wild-type mice, ($n = 5$
962 and 8 mice, respectively).

963 Scale bars in (a), 100 μm ; (e) 5 μm . Data presented as mean $\pm$ SEM. Two-sample Kolmogorov-Smirnov
964 test with Bonferroni correction (b). Wilcoxon rank sum test (c, d, g). Wilcoxon signed-rank test (f).

965

Extended Data 8



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Extended Data Figure 8. A1R agonism suppresses and A1R/P2Y12R blockage enhances neuronal activity while occluding sTREM2 action

(a) Ca^{2+} transient frequency before and during A1R antagonist (DPCPX, 1 μM) bath application in wild-type mice; each dot represents one mouse (n = 6 mice).

(b) Ca^{2+} transient frequency after A1R antagonist bath application (before), during sTREM2 application (5 μM), and 10 min after sTREM2 washout in wild-type mice (n = 6 mice).

(c) As in (a), for $\text{TREM2}^{-/-}$ mice (n = 6 mice).

(d) Normalized activity during A1R antagonist bath application in wild-type and $\text{TREM2}^{-/-}$ mice, relative to baseline (n = 6 mice each).

(e) Representative two-photon image and Ca^{2+} traces from the five labeled neurons before, during, and 10 min after sTREM2 application (5 μM) in P2Y12R antagonist (PSB-0739, 10 μM)-treated wild-type mice.

(f) As in (b), for sTREM2 application in P2Y12R antagonist-treated wild-type mice (n = 6 mice).

(g) Ca^{2+} transient frequency before, during, and after cAdo (0.5 μM) application in wild-type mice; each dot represents one mouse (n = 6 mice).

(h) Ca^{2+} transient frequency before and during bath application of the ENT1 inhibitor dipyridamole (ENT1-i, 20 μM) in wild-type mice (n = 6 mice).

(i) As in (b), for sTREM2 (5 μM) application in ENT1-i-treated (20 μM) wild-type mice (n = 6 mice).

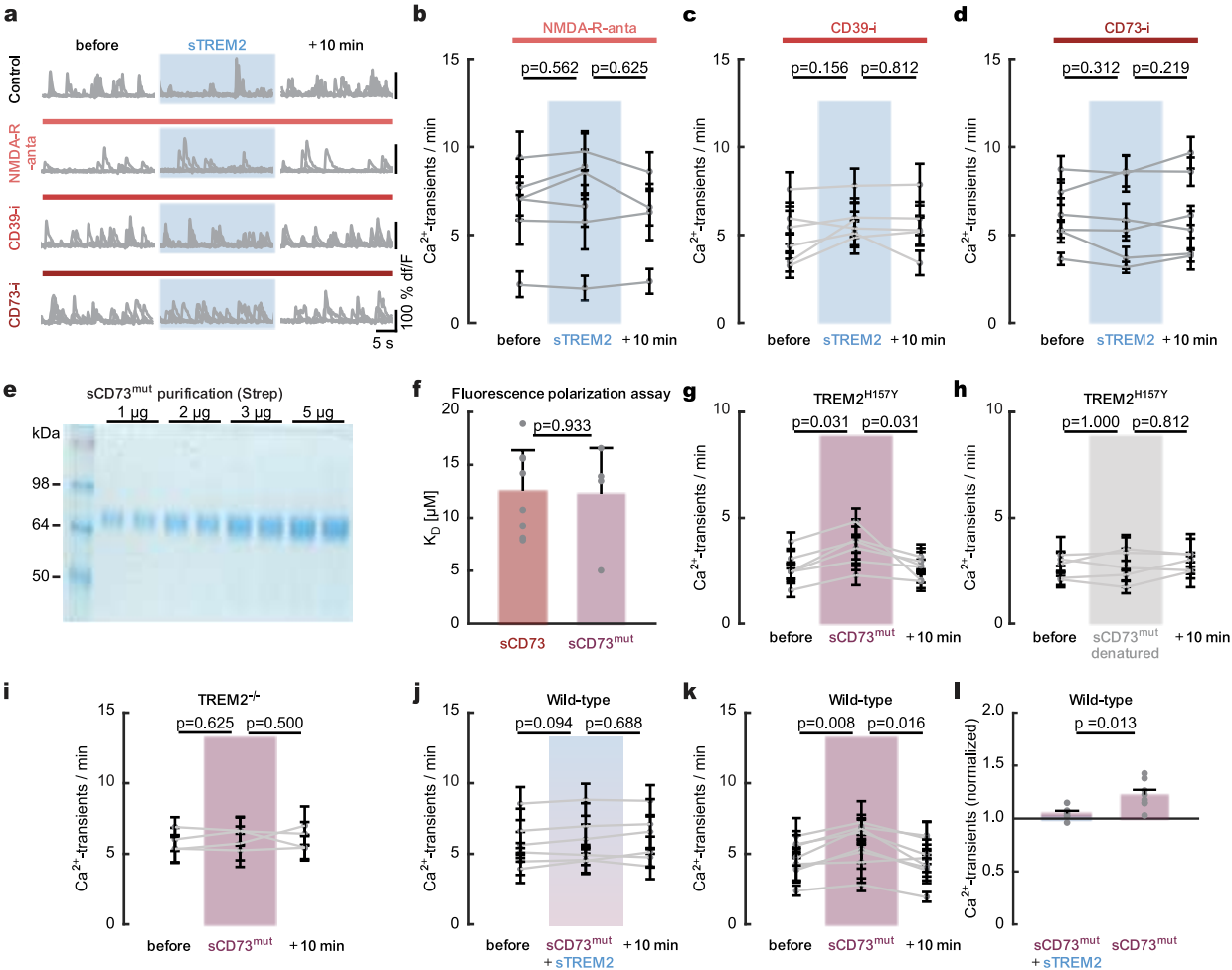
(j) Normalized activity during sTREM2 application (5 μM) in ENT1-i-treated and control wild-type mice (n = 6 and 8 mice, respectively).

(k) As in (g), for adenosine (20 μM) application in microglia-depleted mice (n = 6 mice).

(l) As in (g), for cAdo (0.5 μM) application in $\text{TREM2}^{-/-}$ mice (n = 6 mice).

Scale bar in (e), 5 μm . Data presented as mean $\pm$ SEM (a–l). Two-sided Wilcoxon signed-rank test (a–c, f–l, k, i). Two-sided Wilcoxon rank sum test (d, j).

Extended Data 9



Extended Data Figure 9. Quantification and control experiments of CD73-dependent purinergic microglia–neuron signaling required for sTREM2 action

(a) Representative Ca^{2+} traces from CA1 pyramidal neurons before, during, and 10 min after local sTREM2 (5 μM) application under control conditions or after bath application of specific blockers: NMDA-R-antagonist (APV, 100 μM), CD39-inhibitor (ARL67156, 100 μM) and CD73-inhibitor (AOPCP, 100 μM).

(b) Ca^{2+} transient frequency after NMDA-R antagonist (APV, 100 μM) bath application, before, during sTREM2 application (5 μM), and 10 min after sTREM2 washout in wild-type mice ($n = 6$ mice).

(c) As in (b), for CD39 inhibitor (ARL67156, 100 μM)-treated mice.

(d) As in (b), for CD73 inhibitor (AOPCP, 100 μM)-treated mice.

(e) Coomassie-stained SDS-PAGE showing ascending concentrations of purified, enzymatically inactive sCD73^{mut} (1-2-3-5 $\mu\text{g}/\text{lane}$).

(f) K_D values from fluorescence polarization experiments using fluorescein-labeled sCD73 and sCD73^{mut} ($n = 8$ and 4 independent experiments).

(g) Ca^{2+} transient frequency before, during, and 10 min after sCD73^{mut} (10 μM) application in TREM2^{H157Y} mice; each dot represents one mouse ($n = 6$ mice).

(h) As in (g), for heat-denatured sCD73^{mut} (10 μM) in TREM2^{H157Y} mice ($n = 5$ mice).

(i) As in (g) for sCD73^{mut} (10 μM) in TREM2^{-/-} mice ($n = 4$ mice).

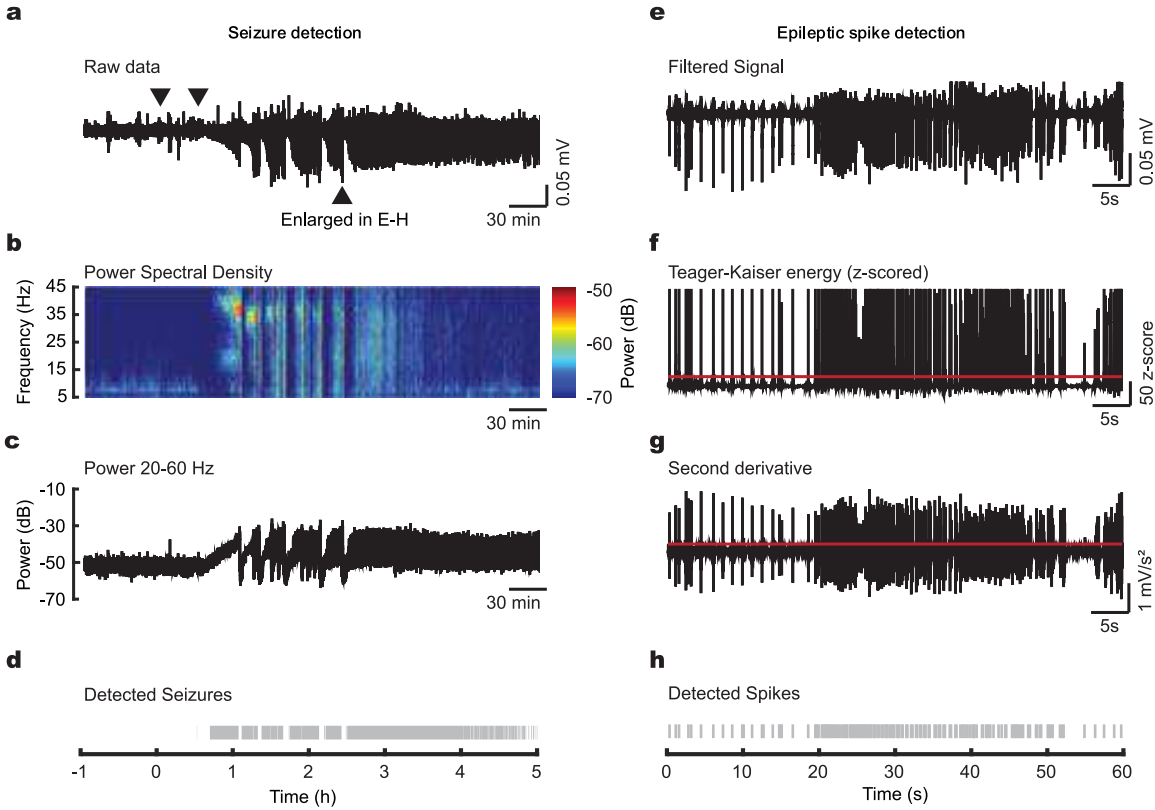
(j) as in (g) for sCD73^{mut} (10 μM) preincubated with sTREM2 (5 μM) in wild-type mice ($n = 6$ mice).

(k) as in (g) for sCD73^{mut} (10 μM) in wild-type mice ($n = 8$ mice).

(l) Normalized activity during sCD73^{mut} (10 μM) preincubated with sTREM2 (5 μM) and sCD73^{mut} (10 μM) alone in wild-type mice, relative to baseline ($n = 6$ and 8 mice, respectively).

Data presented as mean $\pm$ SEM. Two-sided Wilcoxon signed-rank test (b-d, g-k), Two-sided Wilcoxon rank sum test (f,l).

Extended Data 10



Extended Data Figure 10. Algorithm for epileptic spikes and seizure detection for EEG recordings

(a) Raw EEG trace before preprocessing. Arrowheads mark kainic acid (KA) injection time points. The black arrow indicates the 60-s segment expanded in (e–h).

(b) For seizure detection, the EEG signal is high-pass and notch filtered, and the power spectral density is computed using a 1-second sliding Hamming window.

(c) Power in the 20–60 Hz band is summed and z-scored relative to the 1-hour baseline preceding the first KA injection.

(d) Time periods with z-scores exceeding 20 are marked as seizures (shaded areas). Events shorter than 5 seconds are excluded, and those separated by less than 10 seconds are merged.

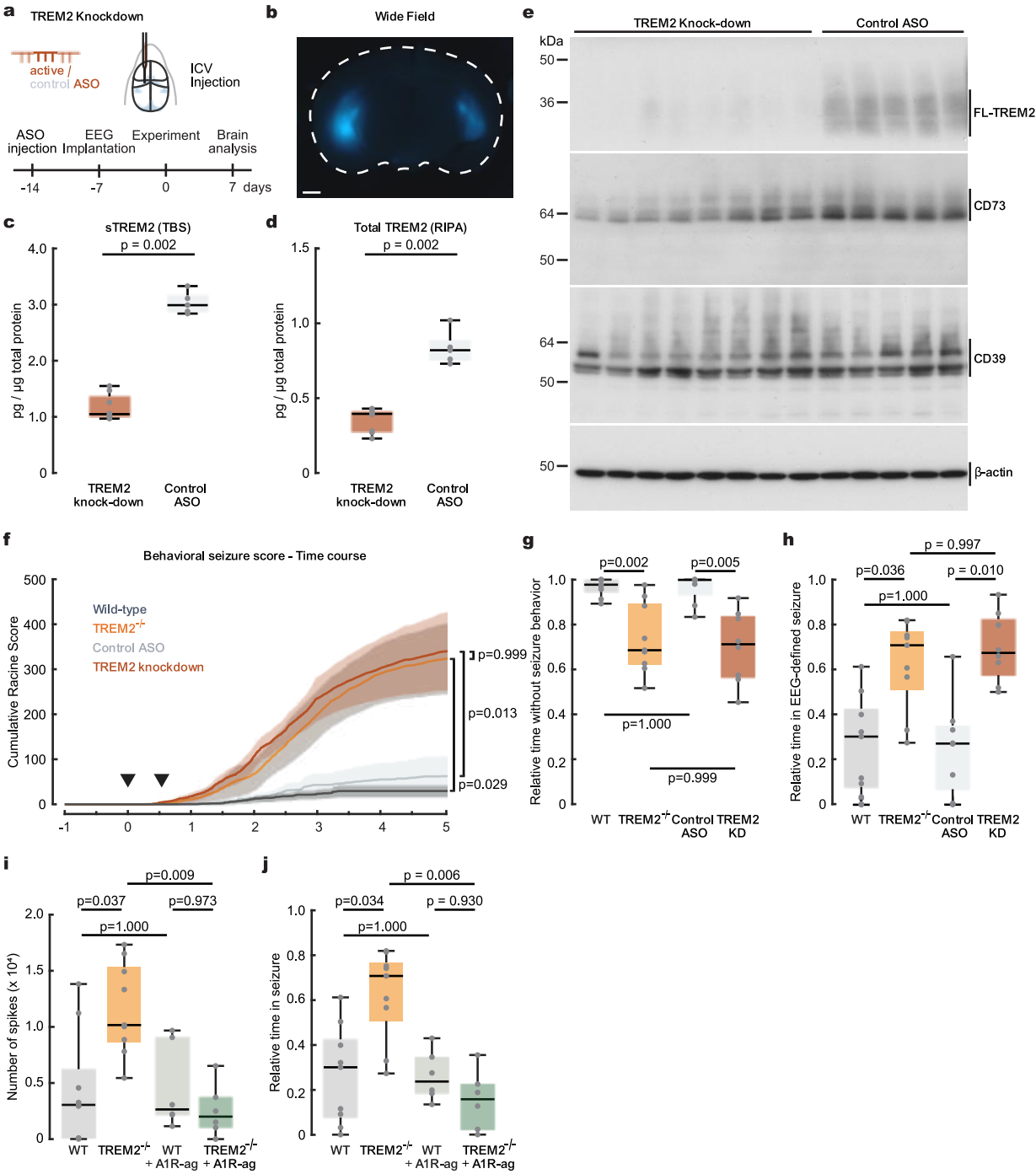
(e) For spike detection, the signal is filtered and artifacts are removed by computing the Teager–Kaiser energy operator (TKEO) on an empty reference channel.

(f) TKEO is applied to the EEG signal and z-scored relative to the pre-KA baseline. The red line indicates the 20 z-score threshold.

(g) The second derivative of the signal is computed to capture sharp transients. The red line marks the threshold of 0.25 mV/s^2 .

(h) Epileptic spikes are detected when both the TKEO z-score exceeds 20 and the second derivative exceeds $250 \text{ } \mu\text{V/s}^2$.

Extended Data 11



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Extended Data Figure 11. Brain-wide knockdown of TREM2 via intracerebroventricular antisense oligonucleotide injection mimics TREM2^{-/-} phenotype

(a) (top) Schematic of intracerebroventricular (ICV) delivery of active or control ASO via stereotactic injection into the lateral ventricle. (bottom) Experimental timeline.

(b) Wide-field image of a brain slice after Dil injection in the lateral ventricle, confirming bilateral distribution.

(c) ELISA-based quantification of sTREM2 in TBS brain lysates from TREM2 knockdown and control ASO mice, sacrificed after the KA challenge shown in Fig. 5d–f (n = 8 and 5 mice, respectively).

(d) As in (c), for total TREM2 levels in RIPA brain lysates.

(e) Western blots of FL-TREM2, CD73, CD39, and β -actin from mice used in the KA challenge experiments.

(f) Cumulative Racine score during the 5-hour recording period for wild-type, TREM2^{-/-}, control-injected and TREM2 knockdown mice (n = 9, 9, 8, and 8 mice, respectively).

(g) Relative time without seizure behavior (Racine score = 0) for the experiment shown in (f).

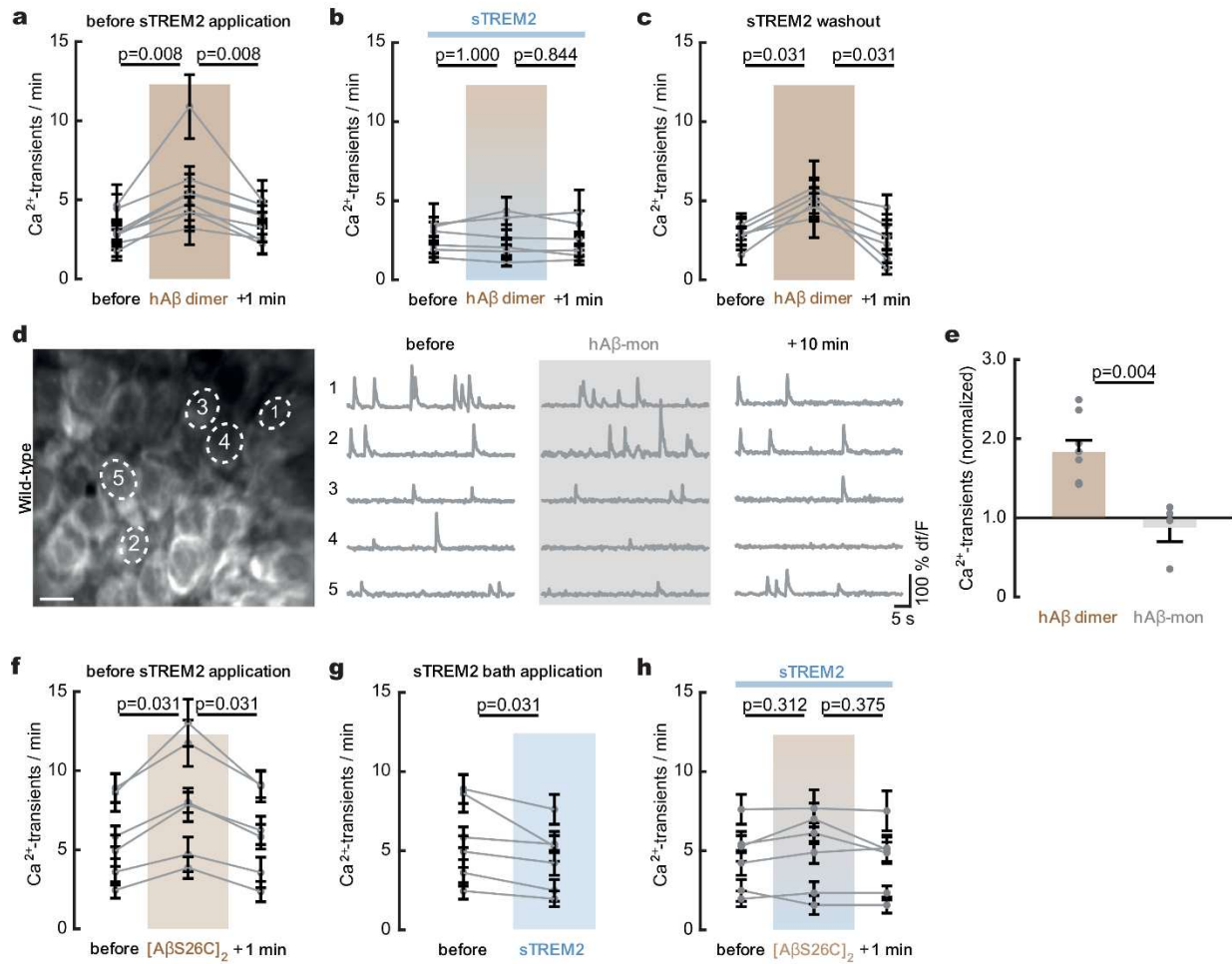
(h) Relative time in EEG-defined seizure for the experiment shown in (f).

(i) Number of epileptic spikes for wild-type and TREM2^{-/-} mice after low-dose KA injections with or without additional A1R agonist (A1R-ag, CCPA, 3 mg/kg body weight injections (n = 9 mice each without A1R-ag; n = 6 each with A1R-ag).

(j) Relative time spent in EEG-defined seizure for the experiment shown in (i).

Scale bar in (b), 1 mm. Data presented as mean $\pm$ SEM (f) and boxplots (c, d, g–j). Two-sided Wilcoxon rank sum test (c, d). Kruskal-Wallis test with Dunn-Sidak post hoc comparison (f–j).

Extended Data 12



Extended Data Figure 12. Additional characterization of sTREM2's effect on human and synthetic Aβ dimer-induced hyperactivation

(a) Ca²⁺ transient frequency before, during, and 1 min after hAβ dimer application (50 ng/ml), prior to sTREM2 bath application, in wild-type mice; each dot represents one mouse (n = 8 mice).

(b) As in (a), for hAβ dimer application during 10 nM sTREM2 bath application (n = 6 mice).

(c) As in (a), for hAβ dimer application after 30-min sTREM2 washout (n = 6 mice).

(d) Representative two-photon image and Ca²⁺ traces from the five labeled neurons before, during, and 10 min after application of human Aβ monomers (hAβ mon, 50 ng/ml) in a wild-type mouse.

1067 (e) Normalized activity during application of hA β dimers or hA β monomers (50 ng/ml each), relative to
1068 baseline (hA β dim, n = 8 mice; hA β mon, n = 4 mice).

1069 (f) As in (a), for synthetic cross-linked A β dimer ([A β S26C]₂, 1 μ M) application, prior to sTREM2 bath
1070 application (n = 6 mice).

1071 (g) Ca²⁺ transient frequency before and during sTREM2 bath application (10 nM; n = 6 mice).

1072 (h) As in (f), for [A β S26C]₂ application during sTREM2 bath application (n = 6 mice).

1073 Scale bar in (d), 5 μ m. Data presented as mean $\pm$ SEM. Two-sided Wilcoxon signed-rank test (a–c, f–h).

1074 Two-sided Wilcoxon rank sum test (e).

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