
The Second Brake

How amyloid- β restrains microglia twice — the monomer at the precursor protein, the plaque at PU.1, and the contradiction that has opened between two laboratories

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Two laboratories have described a brake on the same cell. One found that soluble monomeric amyloid- β holds microglia still through the precursor protein; the other that microglia touching a plaque halve their PU.1 and turn protective. The peptide that supplies the first brake is consumed to build the stimulus for the second — which leaves a region, and an interval, where a microglion may have neither.

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Abstract

Two recent results describe a brake on the same cell. Neither cites the other. Read together they say something neither says alone.

The first is a developmental paper [1]. Working in the embryonic mouse cortex, it reports that the monomeric form of amyloid- β acts on microglia through the amyloid precursor protein and the heterotrimeric G-protein chaperone Ric8a, and that this pathway suppresses microglial cytokine production. Delete either component from microglia, add an immune stimulus, and the microglia turn on matrix metalloproteinase 9, digest the pial basement membrane, and let neurons escape through the holes into the marginal zone. The peptide the field treats as the toxin is, in its soluble monomeric state, holding the brain's phagocyte still.

The second is a disease paper [2]. It reports that microglia which touch an amyloid plaque downregulate the myeloid master transcription factor PU.1, and that the resulting low-PU.1 cells adopt a lymphoid-like regulatory program — CD28, PD-1, PD-L1 — restrain inflammation, compact plaques, block the spread of injected tau seeds, preserve synapses and long-term potentiation, and extend the lifespan of the mice. Lowering PU.1 by half in microglia reproduces the benefit. The human genetics agrees: a common variant in the 3' untranslated region of *SP11* that lowers PU.1 delays the onset of Alzheimer's disease [3].

The structural claim. Amyloid- β brakes the microglion twice, in two opposite physical states, through two unrelated pathways, on two timescales. As a diffusible monomer it acts through a receptor. As a deposited aggregate it acts through cell contact and a transcription factor. And because aggregation consumes monomer by mass action [4,5], the two brakes are anti-correlated in supply: the first is withdrawn precisely as the second is applied. Whether that handover is clean is a question no one has asked, and it has a spatial form — the field of microglia too far from a plaque to be braked by contact but living in a brain whose soluble pool has already been drawn down — and a temporal form, an interval during which neither brake is fully set.

The contradiction. The PU.1 result is not secure, and the way it is insecure is the most informative thing in this literature. A second laboratory has twice reported the opposite sign. Germline heterozygous knockdown of *Spi1* — a 41% reduction in cortical PU.1 protein, a magnitude indistinguishable from the 50% that helps in the first laboratory's hands — makes amyloid pathology worse [6]. Complete microglia-specific deletion in mature cells makes it worse again, and impairs phagocytosis through Syk, Lyn and Fcgr1 [7]. And in the single comparison where mouse model and direction are matched — PU.1 raised in 5xFAD — the two laboratories disagree: the +54% increase is protective in one laboratory's hands, while the +25% increase is, in the other's, the arm against which benefit is measured rather than a source of it.

This paper's central analytical result is that no two of these experiments differ in only one variable. Dose cannot reconcile them, because the magnitudes collide. What separates every

protective result from every harmful one is not how much PU.1 was changed but *where and when*: benefit appears only when the change is both partial and confined to mature microglia. Either departure — going to complete deletion, or going germline and whole-body — flips the sign. A single cross would settle it, and it has not been done.

The convergence. The route that lowers PU.1 at the plaque runs through SYK and PLC γ 2, downstream of TREM2 and CLEC7A; the first laboratory's brake runs through APP and heterotrimeric G proteins. These are different pathways, but they meet the human genome at the same place. The *PLCG2* P522R hypermorph is protective [8,9], the *PLCG2* M28L loss-of-function allele confers risk [10], and the *SPH*-lowering haplotype is protective [3] — three common or rare alleles, one axis, and PLC γ 2 sits immediately upstream of PU.1 in it. That predicts a specific, cheap, untested epistasis in data that already exist.

The therapeutic reading. Both brakes are non-monotonic, and every clinical failure in this space is an instance of treating a set-point as a switch. Antibodies that bind the amyloid monomer produced nothing where they should have worked best [11]. Antibodies that spare the monomer and clear the aggregate produced small but real effects, and raise soluble A β 42 while doing it [12]. An antibody built to *activate* microglia through TREM2 engaged its target, moved its pharmacodynamic markers, and failed [13]. The object being manipulated in all three cases is a rheostat with an optimum, and the optimum is not at either end.

i. Introduction: Two Results, One Cell

1.1 The papers

In December 2024 the laboratory of Zhen Huang, at the University of Wisconsin–Madison, published in *eLife* an account of why a particular mouse mutant develops holes in the surface of its cerebral cortex [1]. The answer turned out to have nothing to do with neurons. It was a paper about microglia, and about the possibility that the monomeric form of amyloid- β is a physiological restraint on them.

In November 2025 the laboratories of Anne Schaefer and Pinar Ayata, at Mount Sinai and the City University of New York, published in *Nature* an account of a subpopulation of microglia sitting on amyloid plaques and defined not by what they express but by what they have lost — roughly half their normal content of PU.1, the transcription factor that makes a myeloid cell a myeloid cell [2]. These cells are protective, and reducing PU.1 deliberately reproduces the protection.

The papers do not cite each other. They share no authors, no model system, no method, and no obvious topic: one is about the assembly of the embryonic neocortex, the other about the progression of amyloid pathology in a transgenic adult. They share a cell, a peptide, and — this is the point of what follows — a logical structure.

1.2 What “brake” is being claimed

The word is used loosely in this literature, so it is worth fixing.

A **brake** in the sense used here is a signal that a tissue supplies to a cell in order to hold that cell below its maximum response. It has three properties. It is *tonic*: present in the healthy state, not summoned by injury. It is *negative*: its removal produces more of something, not less. And its loss is *pathogenic on its own*, without any additional insult beyond those the tissue ordinarily encounters.

The framing is not idiosyncratic. The microglial literature has converged on the idea that a set of pathways — TREM2, CX3CR1, progranulin — act as immune checkpoints holding the inflammatory response in check, and that neurodegeneration follows from an imbalance among sentinel, housekeeping and defence functions rather than from activation as such [14,15].

The distinction matters because the alternative framing — activation as the pathogenic event — has dominated the field and dictates a different therapeutic. If microglia are pathologically activated, the intervention is to suppress them. If microglia are pathologically *disinhibited*, the intervention is to restore an absent restraint, which is a different molecule, a different direction, and,

as sections 9 and 13 argue, a different reason for the failures already on record.

Both papers make brake claims in this strict sense. Huang's is a ligand-and-receptor brake: a peptide binds a receptor and the cell quiets. Ayata's is a transcriptional set-point brake: a dose of a transcription factor determines the gain of the whole inflammatory program. They are not the same kind of object, and the difference is load-bearing.

1.3 Three claims

This paper makes three, of descending confidence.

First, that amyloid- β restrains microglia twice, in two physical states, and that the two restraints are anti-correlated in supply. Aggregation removes monomer; monomer is the first brake's ligand; deposition is the second brake's stimulus. The consequences — spatial and temporal — are set out in section 5. This claim is a synthesis of two published mechanisms plus the polymerisation physics that links them, and it is the paper's principal contribution.

Second, that the PU.1 literature's contradiction is not a dose problem. Section 4 tabulates five experiments across three papers and shows that the magnitudes of the manipulations collide across the sign boundary while the compartment and the developmental timing do not. This is an analytical result about published numbers rather than a hypothesis, and it identifies a decisive experiment that a single mouse cross would supply.

Third, that the human genome has already voted on this axis, three times, in the same direction. Section 7 sets out the *PLCG2*–*SPI1* convergence and derives an epistasis test that could be run on existing genotype data without generating anything new.

Sections 2 and 3 assemble the two mechanisms from their sources and press on the weak points. Section 4 is the contradiction. Sections 5 to 8 develop the synthesis. Section 9 reads the trial record through it. Section 10 grades every substantive claim; sections 11 and 12 state the predictions and the refutation conditions; sections 13 and 14 state the consequences and the limits.

2. The First Brake: The Monomer at the Precursor Protein

2.1 An accident in a developmental screen

The result was not sought. Huang's laboratory was studying *ric8a*, a gene encoding a molecular chaperone required for the post-translational stability of heterotrimeric G-protein α subunits [16,17], in the context of cortical development [18,19]. Deleting it with *emx1-cre* produced neuronal ectopia — clusters of neurons that had migrated past the surface of the cortex and out through breaches in the pial basement membrane — confined to the lateral cortex.

The natural interpretation was a defect in radial glia, the cells whose endfeet anchor that membrane, and which are the standard culprit in cobblestone lissencephaly. The paper's central negative result is that this interpretation fails. Deleting *ric8a* with *nestin-cre*, with *foxg1-cre*, with *wnt3a-cre*, with *nex-cre*, or with *dlx5/6-cre* — covering neural progenitors and their progeny, Cajal–Retzius cells, excitatory neurons and inhibitory neurons — produced no ectopia at all. Radial glial identity, proliferation and mitotic spindle orientation were unaffected. And where classical cobblestone models show radial fibres *retracting* from the breach, here the fibres extended through it, which is the wrong sign for an adhesion defect.

There was also a clue in the phenotype's address. Ectopia confined to the lateral cortex is the signature of a small set of mutants, and it includes mice lacking all three APP family members, whose cortical dysplasia resembles human type 2 lissencephaly [20]. That family resemblance is what eventually pointed the investigation at APP. It also carries its own warning against a neuronal reading: knocking down APP specifically in cortical neurons during development produces neurons that migrate too *little*, not too far [21] — the opposite sign from the ectopia.

What was left was the cell type that expresses *emx1* and is not neural: microglia. Deletion with the microglia-specific *cx3cr1-cre* raised cytokine secretion and transcription on stimulation but produced no ectopia by itself. Adding an immune stimulus — lipopolysaccharide administered in utero at E11.5 and E12.5 — produced ectopia in 10 of 19 mutants and in none of 32 littermate controls. Two hits were required: a microglion with the brake removed, and an occasion to respond.

2.2 The pathway

Having implicated microglia, the paper asks what normally activates the pathway, and reaches amyloid- β by a chain of reasoning worth stating explicitly, because it is a chain and not an observation. APP has a nanomolar-affinity ligand in A β itself [22]. A β oligomers and fibrils are established as pro-inflammatory [23], while non-fibrillar preparations are not; and monomeric A β had been reported to *suppress* T-cell activation and to reverse paralysis in four models of experimental autoimmune encephalomyelitis [24]. If APP is a receptor and the oligomer is the agonist for inflammation, perhaps the monomer is the agonist for restraint.

Prepared in DMSO to preserve the monomeric conformation, A β 40 suppressed secretion and transcription of a wide panel of cytokines from wild-type microglia, whether they had been stimulated with lipopolysaccharide or with polyinosinic-polycytidylic acid, at concentrations from 50 to 500 nM and with peptide from two commercial sources. In *app*-deleted microglia the suppression was abolished; in *aplp2*-deleted microglia it was preserved, which makes the requirement specific to APP rather than to the gene family. In *ric8a*-deleted microglia the suppression of TNF α and IL-6 was abolished, but the suppression of IL-1 β was not — a dissociation the authors attribute, plausibly, to the inflammasome-dependence of IL-1 β secretion [25], and which implies that Ric8a-dependent G proteins carry only part of the signal that APP transduces.

That APP couples to heterotrimeric G proteins is not new. It was reported in 1993, when APP was shown to complex with Go [26], and has been re-established since in several systems and species [27,28,22]. What is new here is the cell type and the direction: an anti-inflammatory function, in microglia, in vivo.

2.3 The effector: MMP9 and the eaten membrane

The pathway from disinhibited microglion to hole in the cortex runs through a protease. In *ric8a* mutants, gelatin zymography showed MMP9 activity elevated roughly 3.7-fold in the E13.5 cortex, with MMP2 unchanged — an internal control that makes the change specific rather than a loading artefact. Laminin-positive debris was already increased at E12.5, before any breach had formed, which places degradation before rather than after the structural failure.

Three pharmacological results close the loop. Dorsomorphin and S3I-201, inhibitors of pro-inflammatory signalling, suppressed astrogliosis, reduced ectopia number and size, and — administered together — nearly abolished ectopia while returning cortical MMP9 activity to control levels. BB94, a broad-spectrum MMP inhibitor, reduced ectopia number and size. An inhibitor selective for MMP9 and MMP13 did the same. The chain — brake removed, cytokines up, MMP9 up, basement membrane digested, neurons escape — is supported at every link by an intervention.

2.4 The sentence that changed between the preprint and the paper

The reviewed preprint of August 2024 states that the authors describe “the discovery of a novel signaling pathway activated by the monomeric form of A β that plays essential roles in the regulation of microglial activity and the assembly of neocortex during development.”

The version of record, published in December 2024, states that they describe “the discovery of a novel signaling pathway activated by the monomeric form of A β **in vitro** that plays essential roles in the regulation of microglial activity and the assembly of neocortex during **mouse** development **in vivo**” [1].

Three words were added, and they mark the exact boundary of the evidence. The ligand claim is cell-culture. The phenotype claim is animal. Nothing in the paper shows amyloid- β activating this pathway in a living brain. The public review record makes the concession explicit: asked for in vivo data that A β monomers are the relevant ligand, the corresponding author replied that “we currently do not have evidence that in the developing cortex Abeta monomers play a role in inhibiting microglia,” and that the paper’s conclusion is that “an Abeta monomer-activated microglial pathway regulates normal brain development, not that Abeta monomers themselves regulate brain development.”

That is a careful and honest distinction, and it should be carried forward into everything built on this result, including this paper. What is established is that *a pathway which monomeric A β can activate in vitro* restrains microglia in vivo. What is not established is that A β is the endogenous ligand. Other APP ligands exist and are expressed in the embryonic cortex.

2.5 What a developmental result licenses

A reader entitled to be sceptical will ask why an embryonic phenotype bears on a disease of the eighth decade. Three things make the extrapolation reasonable, and one makes it limited.

It is reasonable, first, because microglia are the same lineage throughout life — yolk-sac-derived, PU.1- and Irf8-dependent, self-renewing, and never replaced from the bone marrow under normal conditions [29,30]. Second, because the effector is shared: MMP9 is elevated in the plasma of patients with mild cognitive impairment and with Alzheimer’s disease [31,32], and glial MMP9 released near the vasculature damages the basement membrane in the adult exactly as it does in the embryo [33]. Third, because the logic of a tonic brake does not depend on age; a cell held below maximum by a ligand will exceed maximum when the ligand goes, whenever that happens.

It is limited because the embryonic cortex is not the aged cortex, and the immune stimulus in the mouse was a bolus of bacterial endotoxin rather than whatever chronically provokes microglia in a seventy-year-old. The developmental experiment establishes the existence and the wiring of the

brake. It does not establish its magnitude, or even its persistence, in the adult brain. That is a gap, and it is stated again in section 14.

3. The Second Brake: PU.1 Dosage at the Plaque

3.1 A population defined by a deficit

PU.1, encoded by *SPI1*, is the master transcription factor of the myeloid lineage. Mice lacking it have no macrophages [34]; microglia emerge from erythromyeloid precursors along a PU.1- and *Irf8*-dependent path [30]; and the factor's characteristic mode of action is dose-dependent, with different target sets switched on at different concentrations. That last property is stated in the *Nature* paper itself and is the reason everything downstream is quantitative rather than binary.

Working in the 5xFAD amyloid model, Ayata and colleagues identified within the disease-associated microglial population [35,36] a subpopulation expressing markedly *less* PU.1 than the rest. Four observations establish it as a real and distinct state rather than a staining artefact or a contaminant.

It is spatially specific: the low-PU.1 cells preferentially co-localise with amyloid plaques, in the mouse and in human Alzheimer's tissue, and increase in number as disease progresses. It is metabolically distinct: unlike both non-plaque-associated microglia and PU.1-high plaque-associated microglia, its survival does not depend on CSF1R, the receptor microglia otherwise cannot live without — these cells persist through CSF1R inhibition that clears the rest. It is genuinely microglial: lineage tracing with translating-ribosome-affinity-purification mice confirmed the CSF1R-independent cells as bona fide microglia rather than infiltrating peripheral cells, despite their low expression of canonical identity genes. And it is inducible rather than pre-existing, which is the subject of the next subsection.

3.2 The induction route, in four steps

The paper establishes that plaque contact lowers PU.1 through SYK and PLC γ 2 by a four-step argument, and because this route is the keystone of section 7 it is worth setting out exactly what was done.

Step one, the kinases are necessary. Microglia-specific inactivation of either SYK or PLC γ 2 in 5xFAD mice significantly reduced the number of PU.1-low microglia and abolished their CSF1R-independent survival at plaques.

Step two, receptor ligands are sufficient. Ex vivo microglia exposed to phosphatidylserine/phosphatidylcholine liposomes, which engage TREM2, or to β -1,6-glucan pustulan, which engages CLEC7A, downregulated PU.1 in response to both.

Step three, the intermediate is required. Pharmacological inhibition of phospholipase C attenuated the ligand-induced downregulation.

Step four, the intermediate is sufficient by itself. Direct activation of PLC downregulated PU.1 in the absence of any external ligand.

TREM2 and CLEC7A signalling to SYK, and SYK's control of the disease-associated microglial program and of amyloid phagocytosis, are independently established [37,38]. What is added here is the output: that this pathway's job includes turning down the transcription factor that sets the cell's inflammatory gain.

There is a consequence worth flagging now and developing in section 4.6. Ayata's data show that lowering PU.1 *increases* microglial SYK-PLC γ 2 signalling capacity. The pathway that lowers PU.1 is therefore itself potentiated by low PU.1 — positive feedback, which is how a graded stimulus produces a discrete subpopulation rather than a smooth gradient across all cells.

3.3 What the low-PU.1 cell does

The functional characterisation is unusually complete, and it matters that the benefits are not confined to amyloid.

Lowering PU.1 by roughly half, specifically in microglia and only after four weeks of age, decreased amyloid burden and enhanced plaque compaction. It reduced the spread of human Alzheimer's-derived tau aggregates injected into the dentate gyrus, lowering overall pathogenic tau accumulation. It preserved presynaptic terminals — Bassoon and VGLUT2 — in layer V of the cortex, preserved hippocampal long-term potentiation, and preserved performance in open-field and novel-object-recognition tasks, in animals whose littermates showed deficits in all of these. And it significantly extended the lifespan of the 5xFAD mice.

Transcriptionally, the low-PU.1 cells upregulate lipid metabolism and lysosomal function while downregulating interferon and complement signatures — and the complement arm is the one independently established to drive early synapse loss in these models [39], which supplies a mechanism for the preserved terminals and preserved LTP.

This is the observation that forbids reading “restraint” as “quiescence”, and section 6 is built on it: the protective state is not a microglion doing less of everything. It is a microglion doing less inflammation and *more* clearance.

3.4 The lymphoid borrowing, stated carefully

The paper's headline is that these cells express genes normally associated with lymphocytes: *Cd28*, *Pdcd1* (PD-1), *Cd274* (PD-L1), *Cd5*, *Ctla2a*, *Cd52*, *Lat2* and *Cd72*. CD28 was pursued functionally: it is induced by PU.1 downregulation and by plaque-associated cues, in mouse and in human microglia; and microglia-specific deletion of CD28 in 5xFAD mice increased plaque burden and produced a broad type I interferon response across the microglial population, despite CD28 being expressed by only a small fraction of cells.

Two cautions belong here, neither of which the primary paper obscures but both of which the secondary literature has blurred.

Ctla2a is not *CTLA4*. It is a mouse gene encoding a cathepsin-L-like protein with no human orthologue of the same name, and reading it as the microglial equivalent of the T-cell checkpoint receptor is an error of nomenclature, not a finding.

And CD28 itself diverges between mouse and human at a non-conserved residue that changes its signalling [40]. A protective function demonstrated for mouse microglial CD28 does not transfer to human microglia without being shown there.

3.5 The human genetic anchor

The strongest external support for the direction of the effect is not a mouse. A genome-wide survival analysis of 14,406 Alzheimer's cases and 25,849 controls found that the minor allele of rs1057233, within the 3' untranslated region of *SPI1*, is associated with *delayed* onset of Alzheimer's disease and with *lower* SPI1 expression in monocytes and macrophages; disease heritability was enriched within the PU.1 cistrome [3].

This is a strong result of its kind. It is also, and this is the pivot of the next section, a statement about PU.1 in *all* myeloid cells across an entire lifetime — which is not the same manipulation as lowering PU.1 in microglia alone, in a mature animal, by half.

4. The Contradiction

4.1 Two laboratories, opposite signs

The *Nature* result does not stand alone in the literature. It stands against two papers from the laboratory of Jungsu Kim at Indiana University, both of which report the opposite sign, and the second of which appeared four months after it.

In 2024, *Nature Communications*: knockdown of *Spi1* significantly exacerbated insoluble Aβ levels, amyloid plaque deposition and gliosis, while *overexpression* of *Spi1* significantly ameliorated all of these plus dystrophic neurites. The authors’ conclusion — “activating SPI1 or its downstream pathways may have the potential to protect” — is the exact inverse of the *Nature* paper’s [6].

In 2026, *Neuron*: selective deletion of *Spi1* in microglia increased amyloid deposition, gliosis and dystrophic neurites, decreased the microglial response to plaques, and impaired phagocytosis through Syk, Lyn and Fcgr1; directly activating those three genes rescued the Aβ-uptake defect [7].

A 2026 review has noted the tension and proposed that a partial, plaque-associated reduction may be the reconciling case [41]. That proposal is, in outline, correct and it is credited here. What follows goes further, because “partial” turns out not to survive contact with the published magnitudes.

4.2 The five experiments, tabulated

Study	Direction	Magnitude	Compartment	Onset	Amyloid model	Outcome
Ayata 2025 [2]	PU.1 down	~50% (one floxed allele)	Microglia only	Postnatal, wk 4–6	5xFAD	Protective
Ayata 2025 [2]	PU.1 up	~25% (extra transgene)	Microglia only	Postnatal, wk 4–6	5xFAD	Harmful†
Kim 2024 [6]	PU.1 down	~41% protein (URE deletion, het.)	Germline, all myeloid	Conception	APP/PS1 (L166P)	Harmful
Kim 2024 [6]	PU.1 up	~54% protein (transgenic)	Germline, transgenic	Conception	5xFAD	Protective
Kim 2026 [7]	PU.1 down	Complete (homozygous flox)	Microglia only	Postnatal, wk 7	APPPS1-21	Harmful

† The PU.1-high arm requires a caveat that is developed in §4.3. The primary paper’s abstract states only the lowering result; the harmful direction of the raising result is asserted in the research highlight summarising it [40], and could not be confirmed against the primary text consulted here, in which the PU.1-high mice appear throughout as a comparator arm — including in the survival analysis — without a statement that they are worse than controls.

4.3 Dose cannot reconcile them

The intuitive reconciliation — an inverted-U, with a protective optimum somewhere below the normal set-point and harm at both extremes — is the first thing to try and it fails on the numbers.

It fails on the downward arm because the two magnitudes collide. Ayata reduced microglial PU.1 by about 50% and got benefit. Kim reduced cortical PU.1 protein by 40.7% and got harm. Those are not distinguishable quantities. A curve with an optimum between them would have to be implausibly sharp, and would in any case sit in the wrong place relative to Kim’s complete deletion, which is also harmful.

It fails again on the upward arm, where the comparison is the cleanest available anywhere in this literature. **The mouse model and the direction of the manipulation are matched — PU.1 raised in 5xFAD — and the two laboratories do not agree.** Kim’s +54% is unambiguously protective. Ayata’s +25% is, at best, not protective: the PU.1-high animals serve throughout as the arm against which the benefit of PU.1-low is measured, and they do not receive it.

How much weight this bears depends on a fact this paper could not establish. The stronger reading — that raising PU.1 by 25% is *harmful relative to control* — is asserted in the research highlight summarising the *Nature* paper [40], but the paper’s own abstract claims only the lowering result, and the primary text consulted here does not state it. If the PU.1-high arm is merely non-protective rather than harmful, the contradiction on this arm softens from “opposite signs” to “one laboratory finds a benefit from raising PU.1 that the other does not” — which still cannot be reconciled by dose, since the laboratory reporting benefit used the larger increase, but is a weaker fact than the downward arm supplies. The downward arm does not depend on this and is unaffected.

4.4 What does separate them

Set dose aside and read the table again by column. Two variables sort the outcomes perfectly.

Every protective result in the table comes from a manipulation that is **confined to microglia and imposed on a mature cell** — or, in Kim’s overexpression case, from the opposite manipulation applied germline. Every harmful result departs from Ayata’s configuration in exactly one of two ways: it goes to *complete* deletion while remaining microglial and postnatal (Kim 2026), or it stays partial but goes *germline and whole-body* (Kim 2024).

Stated as a rule: **benefit from lowering PU.1 requires that the reduction be both partial and restricted to mature microglia. Either departure inverts the sign.**

This is a stronger and more specific claim than “partial reduction helps”, and it has a mechanistic reading in each direction.

It also has a precedent one node away in the same pathway. Partial and complete loss of TREM2 have been shown to produce *differential* — not merely graded — effects on the microglial injury response and on tauopathy [42]. A field that has already accepted dose-discontinuity at TREM2 should not find it surprising at PU.1.

The **compartment** term is not exotic. PU.1 is a myeloid factor, not a microglial one. A germline URE deletion lowers it in monocytes, macrophages, neutrophils and dendritic cells as well, across the whole of peripheral immunity, in an animal whose brain pathology is known to be sensitive to peripheral immune state. There is no reason to expect the peripheral and central consequences of the same reduction to have the same sign, and the human genetic result [3] — which is also a whole-body, whole-lifetime reduction, and is protective — shows that the peripheral term is not trivially harmful either. It shows that the *net* of the two is protective in humans, which is a third thing again.

The **ontogeny** term is sharper. PU.1 is the factor that specifies the microglial lineage [34,30]. Reducing it from conception is a lesion of identity: the cell that results has been built differently. Reducing it at four to seven weeks, after the identity program has run, is a modulation of state in a cell that is already what it is. Ayata and colleagues say as much about their own design: the changes in PU.1 level were induced postnatally between four and six weeks, in their words, *to avoid developmental confounds*. The confound they took care to avoid is present, unavoidably, in a germline model. These are not the same experiment even at the same percentage, and the field’s habit of describing both as “*Spi1* knockdown” conceals the difference.

4.5 What has not been done

The experiment that would decide this is a single cross away and has not been reported: **a microglia-specific, postnatally induced, heterozygous deletion — Ayata’s dose and timing — carried in Kim’s APPPS1-21 model, with Kim’s readouts.**

Kim 2026 already has the *Cx3cr1*-CreERT2 line, the floxed *Spi1* allele and the amyloid model; the only change required is to score heterozygotes alongside the homozygotes rather than only the latter. Symmetrically, Ayata’s PU.1-low mice could be phenotyped for guanidine-extracted insoluble A β and dystrophic neurites, the readouts on which Kim reports harm and Ayata is silent.

Until one of those is done, the residual possibility that this is not a biology difference at all but a model difference — 5xFAD against APPPS1-21, compaction and burden against insoluble load —

cannot be excluded, and section 10 grades the reconciliation accordingly.

4.6 The feedback that makes a threshold plausible

There is one mechanistic observation that makes a genuinely non-monotonic dose–response likely on independent grounds, and it comes from putting the two laboratories’ molecular findings side by side rather than their phenotypes.

Ayata reports that lowering PU.1 **increases** microglial SYK–PLC γ 2 signalling capacity. Kim reports that deleting *Spi1* **impairs phagocytosis through Syk, Lyn and Fcgr1** — that is, PU.1 is required to express Syk.

Both can be true, and together they describe a system with a floor. PU.1 is required, permissively, for the cell to possess the SYK machinery at all; above some threshold that requirement is saturated, and further PU.1 acts as a constraint on the machinery’s output. Reduce PU.1 partially and the constraint lifts while the requirement stays met — SYK signalling capacity rises, the plaque-contact brake engages more readily, and the positive feedback of section 3.2 carries the cell into the low-PU.1 state. Reduce it to zero and the permissive requirement fails: there is no SYK to potentiate, phagocytosis collapses, and the cell is both uninformed and incapable.

Call these the **permissive floor** and the **regulatory ceiling**. The protective window lies between them, and its width is an empirical quantity nobody has measured. This is an inference, not a finding — it is assembled from two papers that did not measure the same thing — and it is graded as such in section 10. But it is a testable one: SYK protein should be a non-monotonic function of PU.1 dose, rising as PU.1 falls to somewhere near half and then collapsing as PU.1 approaches zero. A titration series in cultured microglia would settle it in a week.

5. One Peptide, Two Brakes, Two Physical States

5.1 The link nobody has drawn

The two brakes have nothing in common at the molecular level. One is a peptide binding a type-I transmembrane protein and signalling through heterotrimeric G proteins. The other is a cell-contact event signalling through a tyrosine kinase and a phospholipase to a transcription factor's promoter. Different ligand states, different receptors, different second messengers, different timescales — minutes against days — and different spatial ranges.

What connects them is arithmetic. Both are set by amyloid- β , and they are set by *different physical states of it*, whose abundances are coupled.

The first brake's agonist is the soluble monomer [1]. The second brake's stimulus is the deposited aggregate [2]. And the conversion of monomer into aggregate is a polymerisation: for a given quantity of peptide, forming fibrils and oligomers necessarily depletes the monomer pool, and the kinetics of that depletion have been characterised in detail [4,5].

It follows, with no additional assumption, that **the two brakes are anti-correlated in supply**. The very process that supplies the second brake removes the first.

One caveat belongs here, because it complicates the clean dichotomy on which the rest of the section rests. The monomer/aggregate distinction is not strictly a property of the extracellular milieu: CD36 mediates an endocytic route that converts soluble ligands, amyloid- β among them, into particulate form *inside* the macrophage, where they disrupt lysosomes and activate the NLRP3 inflammasome [43]. A microglion can therefore manufacture the pro-inflammatory state from the anti-inflammatory species. This does not defeat the anti-correlation argument, which concerns bulk pools, but it means that “monomer present” is not the same as “monomer acting as monomer” at any given cell.

A second caveat is heavier, and the rest of this section should be read under it. The first brake has a floor that has been measured, and an operating range that has not. Monomeric A β 40 suppressed microglial cytokine output at 50, 200 and 500 nM, with significant suppression of TNF α and IL-6 at the lowest concentration tested [1] — and no concentration below 50 nM was tried. Interstitial-fluid amyloid- β in the healthy rodent brain, measured by microdialysis, sits in the low nanomolar range at most [44], and free monomer below that. The demonstrated floor of the first

brake is therefore at or above the whole physiological range, and the concentration at which it engages is also, on the neuronal side of this literature, the concentration at which amyloid- β begins to depress synaptic transmission — the trophic effects that the same peptide exerts on synapses occur three orders of magnitude lower, at 100 to 200 pM [45].

This does not touch the finding, which is a clean piece of cell-culture genetics. It constrains what can be built on it. An argument about a brake being *withdrawn* presupposes that the brake was set, and the anti-correlation of §5.1, the field of §5.3 and the interval of §5.4 all assume a braking threshold that the falling monomer pool crosses on its way down. If the threshold in fact lies above the concentrations a brain contains, there is nothing to withdraw and the handover has no first term. Three readings remain open: the in vivo effect operates below 50 nM at concentrations nobody has tested, or it operates in microenvironments — a synaptic cleft, the surface of a secreting terminal — where local concentration far exceeds bulk interstitial fluid, or it does not operate. The experiment that separates them is a dose-response run downward rather than upward, and it has not been done. Section 12 records this as the synthesis's first failure mode and §10 grades every claim that depends on it accordingly.

5.2 Why this is not merely elegant

The human biomarker literature supplies the independent evidence that the monomer really does fall, and that the fall matters clinically.

Cerebrospinal fluid A β 42 in Alzheimer's disease is roughly half that of healthy individuals, and falls further at the onset of dementia in the genetic forms [12]. Among amyloid-positive individuals — people who have the plaques — *high* soluble A β 42 is associated with normal cognition, in sporadic disease [46] and longitudinally in carriers of *APP*, *PSEN1* and *PSEN2* mutations [47]. This is the “soluble amyloid- β consumption” reading [48]: the amyloid-positive brain that retains its soluble peptide retains its cognition.

That literature was assembled to make a point about neurons — that monomeric A β 42 supports synaptic function and behaves like a neuropeptide, a case with independent support [49,45]. The first brake adds a second beneficiary. If the monomer is also holding microglia still, then the falling soluble pool withdraws a restraint from the brain's phagocyte at the same time as it withdraws a trophic signal from the synapse, and the correlation between preserved soluble A β 42 and preserved cognition acquires a glial term it has not been given.

5.3 The spatial statement

The two brakes do not cover the same territory, and the mismatch is geometric.

The second brake requires **contact**. Ayata's low-PU.1 cells are defined by co-localisation with plaques; the inducing ligands are presented on the deposit and on the membranes around it; the

state does not appear in distal microglia. Its range is the plaque and its immediate halo — micrometres.

The first brake requires **a soluble concentration**, and a diffusible ligand acts wherever it reaches. Its range is the tissue.

But a growing plaque is a sink. It draws down local monomer most steeply nearest itself, which means that in the immediate vicinity of a deposit the first brake is weakest exactly where the second brake is strongest. Move outward and the second brake fails at the edge of the contact zone, while the first recovers only as fast as the local monomer concentration does — and in a brain whose total soluble pool has already fallen by half, it may not recover to the braking threshold at all.

That defines a region: **beyond the reach of contact, below the concentration for the ligand**. Microglia there have lost the first brake to the global drawdown and never acquire the second because they never touch a plaque. If the synthesis is right, that annulus, not the plaque itself, is where the disinhibited microglion should live.

5.4 The temporal statement: the unbraked interval

The same argument, run along the time axis, is sharper, because the two brakes do not decay and rise on the same schedule.

Soluble A β 42 falls early. It is one of the first biomarker abnormalities in the preclinical period, years to decades before dementia, and it falls as a *consequence* of aggregation beginning. Plaque-associated microglia, by contrast, require plaques of sufficient number and size to recruit and hold them, and the low-PU.1 population is explicitly reported to *increase with disease progression* [2] — it is a late and growing population, not an early one.

So the first brake is subtracted early and gradually; the second is added late and increasingly. Between them there is an interval — potentially long, and potentially covering much of the preclinical and prodromal period — during which the monomeric restraint has substantially gone and the transcriptional restraint has not yet been widely applied.

This is the paper’s principal novel proposal, and it should be labelled as what it is: an inference from two established curves that have never been plotted on the same axis. It is not an observation. Section 5.5 says how it could become one.

5.5 How to look for the interval

The claim is falsifiable with existing tissue and existing methods, which is its main virtue.

In human post-mortem material. Stain for PU.1 — routinely used as a microglial nuclear marker — and quantify per-cell intensity as a continuous variable, not a binary call, across a series

of cases spanning Thal and Braak stages, with distance to the nearest plaque recorded for every cell. The synthesis predicts three things: that the PU.1-low fraction rises monotonically with amyloid stage; that within any case it is steeply distance-dependent; and, critically, that in *early* amyloid cases there exists a substantial population of microglia that are neither PU.1-low nor plaque-adjacent — the unbraked field. A finding that PU.1-low cells are already widespread at the earliest amyloid stages, or that no distance dependence exists, refutes the interval.

In the mouse, with a time course. Measure interstitial-fluid monomeric A β by microdialysis [44] and the PU.1-low fraction in the same animals across the age at which plaques appear. The prediction is a crossing: monomer falling before the low-PU.1 population rises, with a measurable gap. If the two move together, there is no interval.

The one-sentence version. If both brakes are amyloid-set, and amyloid must change state to hand over from one to the other, then there is a window in which neither is fully on — and that window, not the plaque, is where the damage is done.

6. Two Axes, Not One

6.1 An apparent conflict

A careful reader will have noticed a problem. In the developmental paper the disinhibited microglion is *destructive*: it makes MMP9 and digests a basement membrane. In the disease paper the restrained microglion is *protective*: it compacts plaques, clears amyloid, blocks tau spread. Fine so far. But the restrained microglion in the second paper is also doing *more* — more lipid metabolism, more lysosomal function, more phagocytosis — while the disinhibited one in the first is doing more cytokine secretion. If restraint means less, how does the restrained cell clear more?

6.2 The resolution

Inflammation and clearance are separable axes, and the protective state is low on the first and high on the second.

This is not a rescue; it is what both papers report. Ayata's low-PU.1 cells upregulate lysosomal and lipid-handling programs while downregulating interferon and complement signatures. Kim's *Spi1*-deleted cells lose phagocytosis through Syk, Lyn and Fcgr1 while gliosis increases — low clearance *and* high inflammation, the worst corner. And SYK, the kinase at the head of the second brake's induction route, is independently established as the mediator of amyloid phagocytosis and of the disease-associated microglial program [37,38]: it drives clearance and lowers PU.1 in the same stroke.

The two axes also explain why the first brake's removal is destructive rather than merely noisy. Ric8a-dependent heterotrimeric G proteins are not only anti-inflammatory; G-protein signalling is coupled to the phagocytic apparatus generally. A *ric8a*-null microglion is therefore predicted to sit in the same bad corner as a *Spi1*-null one — more cytokine, less clearance — which is precisely the corner from which basement membrane gets eaten and nothing gets cleaned up.

6.3 Where each state sits

State	Inflammatory output	Clearance capacity	Net
Homeostatic microglion, both brakes on	Low	Low (unneeded)	Baseline
PU.1-low, plaque-associated [2]	Low	High	Protective
PU.1-high, plaque-associated [2]	High	Moderate	Harmful
<i>Spil</i> -deleted, complete [7]	High (gliosis)	Low (Syk/Lyn/Fcgr1 lost)	Harmful
<i>ric8a</i> - or <i>app</i> -deleted, stimulated [1]	High	Predicted low	Destructive
CSF1R-inhibited (microglia removed)	Absent	Absent	Not a target

6.4 Against quiescence

The therapeutic corollary is that “calming the microglia” is the wrong objective, and has been the wrong objective for as long as the field has pursued anti-inflammatory strategies in Alzheimer’s disease. The target state is not a quiet cell. It is a cell that has been moved along one axis without being moved along the other — and both brakes described in this paper do exactly that, which is why they are worth studying and why a broad-spectrum immunosuppressant is not a substitute for either.

7. The Genetic Convergence

7.1 Two protective alleles on one axis

This section states the paper’s keystone, and it is short because the argument is short.

The route that applies the second brake runs: plaque ligands $\rightarrow$ TREM2 and CLEC7A $\rightarrow$ SYK $\rightarrow$ **PLC γ 2** $\rightarrow$ **PU.1 down** $\rightarrow$ protective state [2].

The human genome contains a well-attested protective variant at each of the last two nodes.

PLCG2 **P522R** is a rare coding variant that reduces Alzheimer’s risk [8] and is a functional **hypermorph** — it weakly increases enzyme activity, and the authors of that characterisation concluded that “activation of PLC γ 2 and not inhibition could be therapeutically beneficial” [9].

SPI1 **rs1057233** is a common variant that delays onset and **lowers** PU.1 [3].

More PLC γ 2 activity. Less PU.1. These are the two consecutive steps of the same pathway, pushed in the two directions that the mouse work says are protective. The alleles were found by different methods, in different studies, years apart, with no knowledge of the mechanism that now connects them.

7.2 The bidirectional control

The *PLCG2* node is better than a single allele, because it has been tested in both directions in the same paper. *PLCG2* P522R, the hypermorph, attenuates disease in an amyloidogenic mouse model; *PLCG2* M28L, a loss-of-function variant, confers increased Alzheimer’s risk in humans and *exacerbates* plaque burden in mice, with altered phagocytosis and A β clearance. The two variants “bidirectionally modulated disease pathology by inducing distinct transcriptional programs” [10].

A pathway in which the hypermorph protects and the hypomorph harms is a pathway whose output is dose-limiting for the phenotype. That is exactly the property required for the node immediately downstream — PU.1 — to be a rheostat rather than a switch, and it is independent evidence for section 4’s reading.

7.3 TREM2 R47H, read as a failure to apply the brake

TREM2 R47H is among the strongest common-and-rare risk alleles in Alzheimer’s disease [50], and its cellular phenotype has been characterised as a failure of the microglial barrier: *TREM2*-deficient or R47H microglia do not envelop plaques, which stay diffuse and less compact, with more severe neuritic dystrophy and tau hyperphosphorylation around them [51,52]. The R47H variant specifically fails to activate microglia via SYK [37], and its consequences extend past amyloid: in a tauopathy model the R47H variant attenuates tau-induced gliosis and, with it, the microglial contribution to neurodegeneration [53].

Under the present synthesis these observations acquire a single mechanism. *TREM2* R47H does not fail to “activate” microglia in some general sense. It fails to deliver the ligand-to-SYK step that lowers PU.1 — and therefore fails to produce the PU.1-low, high-clearance, low-inflammation state whose job is plaque compaction. The compaction defect and the risk allele become the same fact.

This is a reinterpretation, not a new observation, and it is falsifiable: R47H carriers, and *Trem2* R47H knock-in mice, should have a reduced PU.1-low fraction per plaque.

7.4 The epistasis test

If *PLCG2* P522R and the *SPI1*-lowering haplotype act through consecutive steps of one pathway, their protective effects should be **sub-additive** — epistatic rather than independent. Two alleles pushing the same rheostat in the same direction cannot each deliver its full solo effect.

This is testable now, on data that already exist, in any of the large Alzheimer’s case–control collections that have both variants genotyped or imputable. Fit the interaction term. The prediction is a negative interaction coefficient: carriers of both do better than either alone, but by less than the sum.

Two outcomes are informative. A significant negative interaction is strong support for the shared-pathway reading. A clean additive fit, adequately powered, argues that the two alleles act through parallel routes and that the mechanistic chain in section 7.1 does not carry the population-level protection. Given P522R’s rarity, power is the obvious obstacle and would need to be reported honestly; a null from an underpowered test establishes nothing.

7.5 What the convergence does not establish

It does not establish direction of causation in humans, because no human data connect the *SPI1* variant to microglial PU.1 levels *at plaques*; the expression evidence is from monocytes and macrophages [3]. It does not establish that P522R's protection runs through PU.1 rather than through the other things PLC γ 2 does. And it inherits the compartment problem of section 4.4 entire: both variants act in all myeloid cells for a whole lifetime, which is the configuration that, in mice, produced Kim's results rather than Ayata's. The convergence is a strong hint about which axis matters. It is not proof of the mechanism proposed for it.

8. The Effector and the Matrix

8.1 The same protease, a lifetime apart

MMP9 is the executioner in the developmental paper, and it is not confined to development.

In the embryo, microglial MMP9 digests the pial basement membrane and neurons escape through the breach [1]. In the adult, MMP9 released from glia and pericytes near the vasculature damages the vascular basement membrane, while activity-dependent release of the same protease from glutamatergic neurons does something else entirely — it promotes dendritic spine expansion and long-term potentiation [33]. Same enzyme, opposite valence, sorted by which cell released it and where.

The clinical signal is present but coarse: MMP-9 activity is increased in mild cognitive impairment [31] and plasma MMP-9 tracks disease severity in Alzheimer’s disease [32]. And the consequences of a compromised vascular basement membrane in this disease are established independently — blood-derived fibrinogen entering the parenchyma drives microglia-mediated spine elimination and cognitive decline through CD11b [54].

8.2 The cell-source problem, stated plainly

The developmental paper does not establish that microglia are the source of the MMP9 it measures. Asked directly, the authors reported in situ hybridisation showing MMP9 most highly expressed in macrophage-like cells in cortical blood vessels at E12–E13, and inferred that these are microglial-lineage cells populating the embryonic cortex [55] — but that evidence was promised for revision rather than presented. A reviewer separately objected that *Mmp9* message is low or absent in the cortex at E13.5–E14.5 in public expression atlases, casting doubt on the antibody used for the protein staining.

The zymography is the stronger leg: it measures activity, uses equal protein loading, and shows MMP9 up with MMP2 unchanged in the same gel. The functional rescue with two independent inhibitor classes is stronger still. What is genuinely uncertain is not whether MMP activity matters but which cell makes it, and the honest statement is that the paper demonstrates a requirement for MMP9/13 activity without demonstrating its source.

8.3 Why the matrix belongs in this argument

Because it is the tissue-level output that both brakes ultimately protect. A microglion that has lost its restraint does not merely secrete cytokines into a void; it releases proteases into an extracellular matrix that is load-bearing — for the basement membrane in the embryo, for the neurovascular unit in the adult. The synthesis of this paper predicts that the unbraked field of section 5.3 is where matrix damage should be concentrated: not at the plaque core, where the second brake is applied and the cells are compacting rather than digesting, but in the surrounding territory. That prediction is stated formally in section 11.

9. Reading the Trial Record Through Two Brakes

9.1 Antibodies that bind the monomer

Solanezumab binds monomeric A β . In A4, the largest and best-designed prevention trial ever run in preclinical Alzheimer's disease — amyloid-positive, cognitively unimpaired participants, treated for four and a half years — it did not slow cognitive decline [11].

Under a straightforward toxicity model, a monomer-binding antibody in the preclinical window should have been the best shot anyone has taken: intervene early, sequester the species that seeds everything downstream. It did nothing.

Under the two-brake reading, it did something worse than nothing and the trial could not have shown it. A monomer-binding antibody removes the first brake's ligand, in exactly the population — preclinical, few plaques — where the second brake has not yet been applied. That is the unbraked interval, administered pharmacologically. The prediction is not merely null but adverse, and A4 was not designed to detect an adverse effect on microglial state.

This reading is not new in its therapeutic conclusion, which the Huang programme stated years before the readouts [56,57]. What is new is a second, glial reason for it.

9.2 Antibodies that spare the monomer

Lecanemab and donanemab preferentially engage aggregated species — protofibrils and deposited plaque — and both produced statistically robust, clinically modest slowing [58,59]. The contrast with the monomer-binding class is the single most consistent structure in the anti-amyloid trial record.

There is a further observation that the two-brake reading predicts and that has been made independently: the apparent cognitive benefits of anti-amyloid monoclonal antibodies are accompanied by *increased* soluble A β 42 [12]. Removing the aggregate shifts the polymerisation equilibrium back toward monomer. On the two-brake account, an antibody that dissolves plaque is doing two things at once — retiring the second brake's stimulus and restoring the first brake's ligand — and the net benefit depends on the exchange rate between them.

That is a real prediction with an uncomfortable edge, and it should be stated rather than smoothed: if plaque removal retires the second brake, then in a patient far enough along to depend

on it, aggressive clearance could remove more restraint than it restores. Amyloid-related imaging abnormalities are a vascular-matrix phenotype, and section 8 supplies a mechanism by which a locally disinhibited microglion could contribute to one. This is speculation. It is testable speculation: the PU.1-low fraction should fall after plaque clearance, and ARIA-prone tissue should be examinable for it.

9.3 The antibody that engaged its target and failed

AL002 is a TREM2 agonist antibody. Its phase 2 trial in early Alzheimer's disease, in 381 participants over 48 to 96 weeks, demonstrated sustained central target engagement — reduced CSF soluble TREM2, increased osteopontin — and did not meet its primary endpoint on CDR-SB at any of three doses. The most frequent adverse events were imaging changes resembling ARIA [13].

This is the most informative negative trial in the field, because the usual excuse is unavailable. The drug reached the target and moved pharmacodynamic markers. What failed was the hypothesis.

The two-brake reading offers a specific diagnosis. The therapeutic goal was framed as *activating* microglia. But TREM2's protective contribution, on the analysis of section 7.3, is not activation in general — it is the delivery of a particular signal, through SYK to PLC γ 2 to lowered PU.1, that produces a low-inflammation, high-clearance cell. An agonist antibody that engages the receptor and drives the general activation program without reaching that specific output would look exactly like this: engaged, pharmacodynamically active, clinically inert, with a matrix-related adverse event.

If that is right, the tractable handle is not the receptor but the arm. There is precedent: systemic administration of an anti-CLEC7A antibody, which directly activates SYK, rescued microglial activation in mice carrying the TREM2 R47H variant [37].

9.4 Alternatives that must be granted

Three readings compete with the above and cannot be excluded here.

The trials may simply have been too late: by the time of clinical presentation, enough neurons are lost that no microglial manoeuvre helps. The endpoints may be too coarse: CDR-SB over 96 weeks is a blunt instrument for a mechanism that acts on rates. And the anti-amyloid class difference may have nothing to do with monomers — lecanemab and donanemab remove far more amyloid mass than solanezumab did, and a simple dose-of-target-engagement account explains the same pattern without any brake.

That third alternative is the serious one, and it is not defeated by the data assembled here. What the two-brake reading adds is that it also explains the *direction* of the soluble-A β 42 change [12], the

failure of a target-engaged microglial agonist [13], and the human genetics of section 7 — which the mass-of-amyloid-removed account does not address.

10. Strength of Evidence

Every substantive claim in this paper is graded below. **Established** means multiple independent laboratories, or a single result with internal replication and an intervention. **Supported** means one good primary result not yet independently replicated. **Inferred** means assembled here from published parts, with no experiment addressing the composite. **Speculative** means consistent with the evidence and not tested by it.

#	Claim	Grade	Load-bearing evidence	What would move it
1	Microglial <i>ric8a</i> or <i>app</i> deficiency plus immune stimulus causes cortical ectopia via MMP9	Supported	Cell-type-specific <i>cre</i> panel; two inhibitor classes rescue; zymography with internal MMP2 control [1]	Independent replication; identification of the MMP9 source cell
2	Monomeric A β suppresses microglial cytokine output through APP	Supported (in vitro)	Abolished in <i>app</i> -null, preserved in <i>aplp2</i> -null; two peptide sources; two stimuli, at 50-500 nM [1]	An in vivo demonstration; the authors concede none exists. A dose-response below 50 nM
3	A β monomer is the endogenous ligand of this pathway in brain	Speculative	Nanomolar APP binding [22]; monomer anti-inflammatory elsewhere [24]	Conditional removal of the ligand, not the receptor
4	Ric8a carries only part of APP's anti-inflammatory signal	Supported	IL-1 β suppression survives <i>ric8a</i> loss; TNF α and IL-6 do not [1]	Mapping the Ric8a-independent arm
5	Plaque contact lowers PU.1 through SYK and PLC γ 2	Established	Necessity (SYK-KO, PLC γ 2-KO), sufficiency (two ligands), requirement and sufficiency of PLC [2]	—
6	PU.1-low plaque microglia are protective in 5xFAD	Supported	Burden, compaction, tau seeding, synapses, LTP, behaviour, lifespan [2]	Independent replication at matched dose and timing
7	Lower PU.1 delays human Alzheimer's onset	Established	14,406 cases / 25,849 controls; cistrome heritability enrichment [3]	—

#	Claim	Grade	Load-bearing evidence	What would move it
8	Complete or germline <i>Spi1</i> reduction worsens amyloid pathology	Established	Two models, two papers, one laboratory [6,7]	—
9	The contradiction is not explained by dose alone	Established (analytical)	41% harmful vs 50% protective on the downward arm; the larger increase is the protective one on the upward arm (§4.3)	Nothing; this is arithmetic on published numbers
10	Benefit requires the reduction be both partial <i>and</i> restricted to mature microglia	Inferred	Perfect sorting of five experiments on two columns (§4.4)	The missing cross of §4.5
11	A permissive SYK floor and a regulatory PU.1 ceiling bound a protective window	Inferred	SYK capacity up at −50% [2]; Syk expression lost at −100% [7]	A PU.1 titration series measuring SYK protein
12	The two brakes are anti-correlated in supply	Inferred	Polymerisation kinetics [4,5] applied to two established mechanisms; assumes a braking threshold inside the physiological range (§5.1)	Simultaneous measurement of ISF monomer and PU.1-low fraction
13	An unbraked interval exists in the preclinical period	Speculative	Early CSF Aβ42 fall [12]; late rise of PU.1-low cells [2]; inherits the threshold assumption of §5.1	The staged post-mortem series of §5.5
14	An unbraked spatial field exists beyond the plaque halo	Speculative	Contact-dependence of brake 2; diffusibility of brake 1	Distance-resolved PU.1 quantification in human tissue
15	Inflammation and clearance are separable axes; the good state is low/high	Supported	Lysosomal and lipid upregulation in PU.1-low cells [2]; phagocytic collapse in <i>Spi1</i> -null [7]; SYK required for phagocytosis [37,38]	—
16	<i>PLCG2</i> and <i>SPI1</i> protective alleles act on consecutive steps of one axis	Inferred	Hypermorph protective [9,8]; hypomorph harmful [10]; <i>SPI1</i> -lowering protective [3]; PLCγ2 upstream of PU.1 [2]	The epistasis test of §7.4
17	TREM2 R47H is a failure to apply the second brake	Inferred	R47H fails to signal via SYK [37]; compaction defect [51,52]	PU.1-low fraction per plaque in R47H carriers

#	Claim	Grade	Load-bearing evidence	What would move it
18	Monomer-sparing antibodies outperform monomer-binding ones	Established	A4 null [11] vs two positive trials [58,59]	—
19	That difference is <i>because of</i> the monomer	Speculative	Direction of soluble A β 42 change [12]	A trial arm that raises monomer without clearing plaque
20	AL002 failed because it drove activation rather than the SYK-PU.1 arm	Speculative	Target engagement with clinical null [13]; CLEC7A-SYK rescue precedent [37]	A SYK-arm-selective agent in the same population

Nothing in rows 10 to 14, 16, 17, 19 or 20 has been tested as stated. They are the paper's contribution and they are the parts most likely to be wrong.

II. Predictions

Each is stated so that a specific observation would confirm it and a specific observation would refute it.

P1 — The distance gradient. In human Alzheimer’s cortex, per-cell PU.1 intensity will be a monotonically increasing function of distance from the nearest plaque, with the low-PU.1 fraction concentrated within a definable halo. *Refuted by* a flat distance relation.

P2 — The unbraked field. In early-stage amyloid cases, a substantial fraction of microglia will be neither PU.1-low nor plaque-adjacent, and this fraction will be larger in early than in late stages. *Refuted by* PU.1-low cells being already widespread at Thal 1–2.

P3 — The crossing. In an amyloid mouse followed longitudinally, interstitial-fluid monomeric A β will fall measurably before the PU.1-low population rises. *Refuted by* the two changing together, or by the population rising first.

P4 — The SYK titration. SYK protein in microglia will be a non-monotonic function of PU.1 dose: increased at roughly 50% of normal PU.1, decreased as PU.1 approaches zero. *Refuted by* a monotonic relation in either direction.

P5 — The missing cross. Microglia-specific, postnatally induced, *heterozygous Spi1* deletion in APPPS1-21 will reduce, not increase, insoluble A β — reversing the sign of the homozygous result in the same model. *Refuted by* heterozygotes phenocopying homozygotes.

P6 — The compartment. Restoring PU.1 to normal in *peripheral* myeloid cells while leaving microglial PU.1 low will preserve, not abolish, the protective phenotype. *Refuted by* loss of protection.

P7 — Epistasis. *PLCG2* P522R and the *SPI1*-lowering haplotype will show a significant negative interaction on age at onset. *Refuted by* an adequately powered additive fit.

P8 — R47H. *TREM2* R47H carriers and knock-in mice will show a reduced PU.1-low fraction per plaque relative to matched controls. *Refuted by* an unchanged fraction.

P9 — Matrix damage is peri-, not intra-plaque. Markers of MMP9 activity and basement-membrane degradation will be concentrated in the region beyond the microglial plaque halo rather than at the plaque core. *Refuted by* the inverse distribution.

P10 — The antibody consequence. Plaque-clearing antibody treatment will lower the PU.1-low microglial fraction while raising soluble A β 42. *Refuted by* the fraction being unchanged or

increased after clearance.

12. What Would Refute the Synthesis

The two-brake structure fails if any of the following is shown.

The first brake never engages at a physiological concentration. The monomer arm's demonstrated floor is 50 nM, untested below, against bulk interstitial amyloid- β in the low nanomolar range at most (§5.1). If a downward dose-response shows the suppression failing before the physiological range is reached, and no local microenvironment is found in which the concentration is met, then the first brake is a pharmacological effect rather than a tonic restraint. Everything in §5 falls with it, and §9's reading of the monomer-binding antibodies loses its glial term. This is the synthesis's most vulnerable joint — more so than the magnitude question below, which threatens one section's analysis rather than the structure.

The first brake is not real in vivo. The monomer arm rests on one laboratory and on cell culture, with the in vivo gap conceded on the record (§2.4). A failure to replicate the *ric8a/app* microglial ectopia phenotype, or a demonstration that a different APP ligand is the endogenous agonist, removes the first brake from the argument entirely. The second brake would survive; the synthesis would not.

The second brake is a model artefact. If the *Nature* result fails to replicate at matched dose and timing, or if the PU.1-low state proves specific to 5xFAD, section 4's reconciliation becomes moot and Kim's sign stands.

The magnitudes are not what they appear. The analytical claim of §4.3 depends on the reported reductions — 40.7% of cortical protein against approximately 50% of microglial protein. These are not the same measurement: one is bulk cortex, the other is microglia-specific. If the microglial reduction in the URE-deletion model is substantially deeper than the bulk figure implies, the dose explanation is partly rehabilitated and §4.4's rule weakens accordingly. This is the most vulnerable point in the paper's *analytical* claim — as distinct from its structural one, whose weakest joint is the concentration problem above — and it is why claim 10 is graded *inferred* rather than *established*.

The brakes are not anti-correlated where it matters. The mass-action argument concerns total peptide. If local monomer concentration near plaques is buffered — by continued neuronal secretion, by clearance dynamics, by the plaque acting as a reservoir rather than a sink — then the handover has no gap and there is no interval.

The interval does not exist. If PU.1-low microglia are already abundant at the earliest amyloid stages, the temporal claim fails on its own terms.

13. Therapeutic Consequences

13.1 Set-points, not switches

The single practical statement this paper supports is that both restraints described here are rheostats with optima, and that every intervention attempted against them so far has been designed as though they were switches.

Remove the monomer entirely: fails, and should be expected to fail [11]. Delete PU.1 entirely: fails [7]. Activate the receptor maximally: fails, with target engagement documented [13]. Halve PU.1 in the right cell at the right time: works, in one laboratory [2]. The pattern is not subtle, and it is the same pattern the neuronal side of the amyloid literature produced twenty years earlier, where picomolar A β enhances plasticity and nanomolar A β destroys it [45,60].

13.2 The arm, not the receptor

If section 7.3 is right, TREM2 is the wrong level at which to intervene, because the receptor initiates several programs and only one of them is the protective output. The tractable handle is downstream: the SYK–PLC γ 2 arm, where a hypermorphic human allele already demonstrates that a *weak* increase in activity is protective across a lifetime [9], and where a direct CLEC7A-based activator has already been shown to rescue R47H microglia in mice [37].

The design constraint that follows is unusual and should be stated explicitly: the goal is a *weak* agonist. P522R is a mild hypermorph. Ayata's protective manipulation was a 50% change, and a 25% change in the other direction was harmful. Nothing in this literature rewards potency.

13.3 The window problem

The corollary is uncomfortable for drug development. An intervention whose therapeutic window is bounded above by a regulatory ceiling and below by a permissive floor (§4.6), whose optimum may differ between microglia and peripheral myeloid cells (§4.4), and whose target state is late-arriving and plaque-local (§5.4), is not a good candidate for a systemically dosed antibody. It is a candidate for cell-type-restricted delivery with a measurable pharmacodynamic readout of the state itself — the PU.1-low fraction — rather than of receptor occupancy. AL002 measured occupancy and its downstream markers, and had no way to know whether it had produced the cell it wanted [13].

13.4 What not to do

Do not remove the monomer. That principle was stated on other grounds before the trial data arrived [56], and the first brake supplies a second reason for it.

Do not deplete microglia. CSF1R inhibition removes the PU.1-high population preferentially and spares the PU.1-low one [2], which sounds convenient until one notices that it also removes every cell that has not yet reached a plaque — the population that would have supplied the protective state as disease advanced.

Do not treat “reduce neuroinflammation” as the objective. Section 6 is the argument: the protective cell is not the quiet cell.

14. Limitations of This Assessment

The evidence base is asymmetric. Full text was read for the *Nature* paper [2], for both Kim papers [6,7], and for the *eLife* paper together with its complete public peer-review record [1]. Several supporting references — the trial reports in particular — were assessed from abstracts and published summaries rather than from full texts and supplementary data. Where a claim in section 10 depends on a number, that number was taken from the primary paper’s own text.

The first brake’s operating concentration is unknown. The tested range is 50-500 nM and the physiological range is below it (§5.1). This assessment did not resolve the question and does not treat it as resolved; the structural claim of §5 is stated conditionally on it throughout.

One laboratory per brake. The first brake rests on a single laboratory’s work; the second is contradicted by a single laboratory’s work. Neither situation is stable, and this paper’s central analysis is an attempt to make the second one productive rather than to resolve it, which cannot be done from the literature.

One tabulated outcome is second-hand. The direction of the PU.1-high result in the *Nature* paper is taken from the research highlight summarising it [40] and is flagged in the table and in §4.3. Every other entry in that table was read from the primary paper’s own text.

The comparison of magnitudes crosses measurement types. As stated in §12, Kim’s 40.7% is bulk cortical protein and Ayata’s ~50% is microglia-specific. The comparison is the best available and it is not exact.

The synthesis is not a theory of onset. Nothing here explains why amyloid begins to aggregate, and nothing here requires that microglial disinhibition is the initiating event in Alzheimer’s disease. The two-brake structure is a claim about what happens to one cell type as amyloid changes state. It is compatible with several accounts of what starts the process and adjudicates between none of them.

The tau evidence is a seeding experiment. The claim that PU.1-low microglia limit tau spread rests on injection of human Alzheimer’s-derived tau aggregates into the dentate gyrus of 5xFAD mice [2]. That is a strong assay for propagation and a weak one for endogenous tauopathy.

15. Conclusion

Two laboratories, working on different problems, have described restraints on the same cell. One found that the soluble monomeric form of amyloid- β holds microglia below their maximum through the precursor protein, and that removing the restraint lets them digest a basement membrane. The other found that microglia touching an amyloid plaque halve their content of the transcription factor that sets their inflammatory gain, and that this makes them protective.

The peptide that supplies the first brake is consumed to build the stimulus for the second. That is the fact the two papers do not state, because neither is looking at the other, and it converts two unrelated mechanisms into a single structure with a handover in it — one restraint withdrawn diffusely and early, another applied locally and late, and a region and an interval between them where a microglion may have neither.

Whether that gap is real is an empirical question, and section 11 says how to close it. Whether the second brake is real at all is contested, and section 4 argues that the contest has been misread: the disagreement between the laboratories is not about how much PU.1 to remove, because the magnitudes that produce opposite outcomes overlap. It is about *where* and *when*, and one mouse cross separates the hypotheses.

What can be said with more confidence is smaller and more useful. Both of these restraints are graded. Both have optima that are not at either end of their range. The human genome has selected, at two consecutive nodes of one of them, for weak changes in the protective direction — a mild increase in phospholipase activity, a modest decrease in a transcription factor. Every therapeutic attempt in this area has instead applied a maximal change: bind all the monomer, delete the whole gene, saturate the receptor. Each has failed, one of them with its target demonstrably engaged.

The lesson is not that microglia should be suppressed, or activated. It is that they are held, normally, in a narrow band by signals the tissue supplies, and that the useful question is not which direction to push but how far — and that this is a question no trial in this field has yet been designed to ask.

Provenance

This paper is a synthesis of published work. It reports no new experiments. Its contribution is the structural claim of section 5, the analysis of section 4, the convergence of section 7, and the

predictions of section 11.

All citations are PubMed identifiers, expanded from records retrieved directly from PubMed; the manuscript source carries the identifiers rather than the formatted references, and the reference list is generated from the verified records, so that the numbering and the bibliography cannot drift apart.

References

1. Kwon HJ, Santhosh D, Huang Z. A novel monomeric amyloid β -activated signaling pathway regulates brain development via inhibition of microglia. *Elife* 2024;13. PMID 39635981.
2. Ayata P, Crowley JM, Challman MF, et al. Lymphoid gene expression supports neuroprotective microglia function. *Nature* 2025;648(8092):157-165. PMID 41193812.
3. Huang KL, Marcora E, Pimenova AA, et al. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer's disease. *Nat Neurosci* 2017;20(8):1052-1061. PMID 28628103.
4. Dear AJ, Michaels TCT, Meisl G, et al. Kinetic diversity of amyloid oligomers. *Proc Natl Acad Sci U S A* 2020;117(22):12087-12094. PMID 32414930.
5. Michaels TCT, Šarić A, Curk S, et al. Dynamics of oligomer populations formed during the aggregation of Alzheimer's A β 42 peptide. *Nat Chem* 2020;12(5):445-451. PMID 32284577.
6. Kim B, Dabin LC, Tate MD, et al. Effects of SPI1-mediated transcriptome remodeling on Alzheimer's disease-related phenotypes in mouse models of A β amyloidosis. *Nat Commun* 2024;15(1):3996. PMID 38734693.
7. Kim B, Tate MD, Karahan H, et al. Deletion of SPI1 in microglia exacerbates amyloid pathology by impairing microglial response in Alzheimer's disease models. *Neuron* 2026;114(15):2741-2758.e6. PMID 41928507.
8. Sims R, van der Lee SJ, Naj AC, et al. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat Genet* 2017;49(9):1373-1384. PMID 28714976.
9. Magno L, Lessard CB, Martins M, et al. Alzheimer's disease phospholipase C-gamma-2 (PLCG2) protective variant is a functional hypermorph. *Alzheimers Res Ther* 2019;11(1):16. PMID 30711010.
10. Tsai AP, Dong C, Lin PB, et al. Genetic variants of phospholipase C- γ 2 alter the phenotype and function of microglia and confer differential risk for Alzheimer's disease. *Immunity* 2023;56(9):2121-2136.e6. PMID 37659412.
11. Sperling RA, Donohue MC, Raman R, et al. Trial of Solanezumab in Preclinical Alzheimer's Disease. *N Engl J Med* 2023;389(12):1096-1107. PMID 37458272.

12. Espay AJ, Ezzat K, Kepp KP, et al. Restoring amyloid- β 42 and γ -secretase function in Alzheimer's disease. *Brain* 2025;148(11):3856-3864. PMID 40795314.
13. Mummery CJ, Mayorga AJ, Simmons A, et al. The TREM2 agonistic antibody AL002 in early Alzheimer's disease: a phase 2 randomized trial. *Nat Med* 2026;32(5):1708-1716. PMID 41787076.
14. Hickman S, Izzy S, Sen P, et al. Microglia in neurodegeneration. *Nat Neurosci* 2018;21(10):1359-1369. PMID 30258234.
15. Butovsky O, Weiner HL. Microglial signatures and their role in health and disease. *Nat Rev Neurosci* 2018;19(10):622-635. PMID 30206328.
16. Tall GG, Krumins AM, Gilman AG. Mammalian Ric-8A (synembryn) is a heterotrimeric G α protein guanine nucleotide exchange factor. *J Biol Chem* 2002;278(10):8356-62. PMID 12509430.
17. Gabay M, Pinter ME, Wright FA, et al. Ric-8 proteins are molecular chaperones that direct nascent G protein α subunit membrane association. *Sci Signal* 2011;4(200):ra79. PMID 22114146.
18. Ma S, Kwon HJ, Huang Z. Ric-8a, a guanine nucleotide exchange factor for heterotrimeric G proteins, regulates bergmann glia-basement membrane adhesion during cerebellar foliation. *J Neurosci* 2012;32(43):14979-93. PMID 23100420.
19. Ma S, Santhosh D, Kumar T P, et al. A Brain-Region-Specific Neural Pathway Regulating Germinal Matrix Angiogenesis. *Dev Cell* 2017;41(4):366-381.e4. PMID 28535372.
20. Herms J, Anliker B, Heber S, et al. Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. *EMBO J* 2004;23(20):4106-15. PMID 15385965.
21. Young-Pearse TL, Bai J, Chang R, et al. A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. *J Neurosci* 2007;27(52):14459-69. PMID 18160654.
22. Fogel H, Frere S, Segev O, et al. APP homodimers transduce an amyloid- β -mediated increase in release probability at excitatory synapses. *Cell Rep* 2014;7(5):1560-1576. PMID 24835997.
23. Halle A, Hornung V, Petzold GC, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat Immunol* 2008;9(8):857-65. PMID 18604209.

24. Grant JL, Ghosn EE, Axtell RC, et al. Reversal of paralysis and reduced inflammation from peripheral administration of β -amyloid in TH1 and TH17 versions of experimental autoimmune encephalomyelitis. *Sci Transl Med* 2012;4(145):145ra105. PMID 22855462.
25. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016;16(7):407-20. PMID 27291964.
26. Nishimoto I, Okamoto T, Matsuura Y, et al. Alzheimer amyloid protein precursor complexes with brain GTP-binding protein G(o). *Nature* 1993;362(6415):75-9. PMID 8446172.
27. Ramaker JM, Swanson TL, Copenhaver PF. Amyloid precursor proteins interact with the heterotrimeric G protein Go in the control of neuronal migration. *J Neurosci* 2013;33(24):10165-81. PMID 23761911.
28. Milosch N, Tanriöver G, Kundu A, et al. Holo-APP and G-protein-mediated signaling are required for sAPP α -induced activation of the Akt survival pathway. *Cell Death Dis* 2014;5(8):e1391. PMID 25165877.
29. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010;330(6005):841-5. PMID 20966214.
30. Kierdorf K, Erny D, Goldmann T, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. *Nat Neurosci* 2013;16(3):273-80. PMID 23334579.
31. Bruno MA, Mufson EJ, Wu J, et al. Increased matrix metalloproteinase 9 activity in mild cognitive impairment. *J Neuropathol Exp Neurol* 2009;68(12):1309-18. PMID 19915485.
32. Tsiknia AA, Sundermann EE, Reas ET, et al. Sex differences in Alzheimer's disease: plasma MMP-9 and markers of disease severity. *Alzheimers Res Ther* 2022;14(1):160. PMID 36324151.
33. Amontree M, Nelson M, Stefansson L, et al. Resveratrol differentially affects MMP-9 release from neurons and glia; implications for therapeutic efficacy. *J Neurochem* 2024;168(9):1895-1908. PMID 38163875.
34. McKercher SR, Torbett BE, Anderson KL, et al. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. *EMBO J* 1996;15(20):5647-58. PMID 8896458.
35. Keren-Shaul H, Spinrad A, Weiner A, et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* 2017;169(7):1276-1290.e17. PMID 28602351.

36. Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity* 2017;47(3):566-581.e9. PMID 28930663.
37. Wang S, Sudan R, Peng V, et al. TREM2 drives microglia response to amyloid- β via SYK-dependent and -independent pathways. *Cell* 2022;185(22):4153-4169.e19. PMID 36306735.
38. Ennerfelt H, Frost EL, Shapiro DA, et al. SYK coordinates neuroprotective microglial responses in neurodegenerative disease. *Cell* 2022;185(22):4135-4152.e22. PMID 36257314.
39. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 2016;352(6286):712-716. PMID 27033548.
40. Sun C, Liu Q. PU.1: the conductor of Alzheimer's symphony. *Mol Neurodegener* 2026;21(1). PMID 41821106.
41. Chmiel J, Gawęczyk W, Soczyńska J, et al. Lymphoid-like Suppressive Microglia in Alzheimer's Disease: A New Neuroimmune Regulatory Axis? *Cells* 2026;15(13). PMID 42439628.
42. Sayed FA, Telpoukhovskaia M, Kodama L, et al. Differential effects of partial and complete loss of TREM2 on microglial injury response and tauopathy. *Proc Natl Acad Sci U S A* 2018;115(40):10172-10177. PMID 30232263.
43. Sheedy FJ, Grebe A, Rayner KJ, et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. *Nat Immunol* 2013;14(8):812-20. PMID 23812099.
44. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron* 2005;48(6):913-22. PMID 16364896.
45. Puzzo D, Privitera L, Fa' M, et al. Endogenous amyloid- β is necessary for hippocampal synaptic plasticity and memory. *Ann Neurol* 2011;69(5):819-30. PMID 21472769.
46. Sturchio A, Dwivedi AK, Young CB, et al. High cerebrospinal amyloid- β 42 is associated with normal cognition in individuals with brain amyloidosis. *EClinicalMedicine* 2021;38:100988. PMID 34505023.
47. Sturchio A, Dwivedi AK, Malm T, et al. High Soluble Amyloid- β 42 Predicts Normal Cognition in Amyloid-Positive Individuals with Alzheimer's Disease-Causing Mutations. *J Alzheimers Dis* 2022;90(1):333-348. PMID 36120786.

48. Espay AJ, Sturchio A, Schneider LS, et al. Soluble Amyloid- β Consumption in Alzheimer's Disease. *J Alzheimers Dis* 2021;82(4):1403-1415. PMID 34151810.
49. Giuffrida ML, Caraci F, Pignataro B, et al. Beta-amyloid monomers are neuroprotective. *J Neurosci* 2009;29(34):10582-7. PMID 19710311.
50. Guerreiro R, Wojtas A, Bras J, et al. TREM2 variants in Alzheimer's disease. *N Engl J Med* 2012;368(2):117-27. PMID 23150934.
51. Yuan P, Condello C, Keene CD, et al. TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. *Neuron* 2016;90(4):724-39. PMID 27196974.
52. Wang Y, Ulland TK, Ulrich JD, et al. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *J Exp Med* 2016;213(5):667-75. PMID 27091843.
53. Gratuze M, Leyns CE, Sauerbeck AD, et al. Impact of TREM2R47H variant on tau pathology-induced gliosis and neurodegeneration. *J Clin Invest* 2020;130(9):4954-4968. PMID 32544086.
54. Merlini M, Rafalski VA, Rios Coronado PE, et al. Fibrinogen Induces Microglia-Mediated Spine Elimination and Cognitive Impairment in an Alzheimer's Disease Model. *Neuron* 2019;101(6):1099-1108.e6. PMID 30737131.
55. Squarzoni P, Oller G, Hoeffel G, et al. Microglia modulate wiring of the embryonic forebrain. *Cell Rep* 2014;8(5):1271-9. PMID 25159150.
56. Huang Z. A Function of Amyloid- β in Mediating Activity-Dependent Axon/Synapse Competition May Unify Its Roles in Brain Physiology and Pathology. *J Alzheimers Dis* 2023;92(1):29-57. PMID 36710681.
57. Huang Z. Evidence that Alzheimer's Disease Is a Disease of Competitive Synaptic Plasticity Gone Awry. *J Alzheimers Dis* 2024;99(2):447-470. PMID 38669548.
58. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in Early Alzheimer's Disease. *N Engl J Med* 2022;388(1):9-21. PMID 36449413.
59. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in Early Symptomatic Alzheimer Disease: The TRAILBLAZER-ALZ 2 Randomized Clinical Trial. *JAMA* 2023;330(6):512-527. PMID 37459141.
60. Zott B, Simon MM, Hong W, et al. A vicious cycle of β amyloid-dependent neuronal hyperactivation. *Science* 2019;365(6453):559-565. PMID 31395777.