
The Peptide and the Channel

*LL-37, CLIC1, and the Microglial Route from Failed Mitophagy to
cGAS–STING in Alzheimer’s Disease*

Benjamin Aaron Gustafsson

AdultCognitiveDisease.com

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*A graded reconstruction of seven elements into one testable circuit, with every link scored for the
evidence that actually supports it — and every link that does not exist named as such.*

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Abstract

Seven elements are examined here for whether they form a mechanism or merely a list: microglia, the cathelicidin peptide LL-37, the metamorphic protein CLIC1, lysosomal degradative failure, mitophagy, mitochondrial DNA, and the cGAS–STING sensing pathway. They do form a mechanism. The connections are real, they run in a definable order, and the order closes on itself twice. That is the positive claim of this review, and it is made with the specific reservation that no part of the resulting circuit has been demonstrated end to end in human Alzheimer's disease tissue.

The proposed circuit runs as follows. LL-37 is an endogenous agonist of CLIC1 and drives its translocation from cytosol into the microglial plasma membrane, where the inserted protein sustains an oxidative burst and licenses inflammasome assembly. The resulting oxidative and degradative load falls on microglial mitochondria and lysosomes together. Mitochondrial quality control depends on lysosomal completion, so a lysosome that cannot acidify converts mitophagy from a clearance process into a holding pattern. Mitochondrial DNA that is neither degraded nor contained reaches the cytosol, where cGAS and STING convert it into a sustained type I interferon signal. Two returns close the loop: interferon and infection raise transcription of the gene encoding LL-37, and mitochondria-derived reactive oxygen species are themselves a principal trigger for CLIC1 oxidation and membrane insertion. LL-37 also acts on the sensing step directly, carrying DNA across the membrane to the cytosolic sensor and shielding mitochondrial DNA from nuclease attack.

Every link is graded on a five-level scale from direct human disease evidence to untested prediction, and the grading is unflattering to the model. The strongest segments — mitochondrial DNA to cGAS–STING, and interferon to synapse loss — are supported in human tissue and in animal models. The segment on which the whole circuit turns, lysosomal acidification failure stalling mitophagy in Alzheimer's disease, has been asserted for a decade and never directly measured. The peptide-to-channel link rests on a single unreplicated study. The most-cited demonstration that failed mitophagy activates STING has been retracted. Ten specific relationships between these elements return no primary literature at all, and they are named.

Two serious problems are given their own treatment rather than a footnote. The first is that the same peptide is placed at opposite ends of the same disease by two credible research programmes, one holding that excess LL-37 drives pathology and the other that its destruction permits pathology. The second is that cell-free mitochondrial DNA is reported as reduced, not elevated, in preclinical Alzheimer's disease, which is the opposite of what a leakage model predicts. A biphasic reading is proposed for the first and an explicit falsification condition is offered for the second.

The review closes with numbered predictions and a four-stage experimental programme ordered so that the cheapest and most decisive measurement — whether these molecules are present in human Alzheimer's brain at all — comes first, and so that a negative result at any stage terminates the rest.

1. Seven Terms and One Cell

1.1 The question a list of molecules does not answer

A list of molecules associated with a disease is not a mechanism. It becomes one only when the elements can be placed in an order, when each step can be perturbed independently of the others, and when the consequences of perturbation follow the order rather than the list. Alzheimer's disease research has accumulated a very large number of associated molecules and a much smaller number of orders.

The seven elements examined here were not chosen because they are individually prominent. They were chosen because there is reason to think they belong to one another. Microglia are the cell in which six of the other elements are simultaneously present. LL-37 is a cationic host-defence peptide with an unusual property: it binds DNA and changes what happens to that DNA. CLIC1 is a protein with no single structure, which converts between a soluble cytosolic form and an inserted membrane form in response to oxidation. Lysosomal degradative capacity is the terminal step of both autophagy and phagocytosis. Mitophagy is the specific quality-control route that depends on that terminal step. Mitochondrial DNA is the cargo that route is meant to destroy. cGAS–STING is the sensor that detects that cargo when it escapes.

Stated that way the arrangement is close to a syllogism, and should be distrusted for exactly that reason. Plausible arrangements of real molecules are easy to construct and are the characteristic failure mode of mechanistic reviews. The purpose here is therefore not to present the arrangement but to grade it — to say, for every step, what kind of evidence supports it, in what organism, in what cell, and whether anyone has looked.

The grading is uneven in an instructive way. Some links are supported by human post-mortem tissue and by genetic manipulation in animals. Some are supported only in macrophages or keratinocytes and are transferred to microglia on the assumption that a myeloid cell is a myeloid cell. Some rest on a single laboratory and have not been reproduced in four years. One central link — the one that makes the arrangement a circuit rather than two unrelated halves — has never been measured in brain tissue. And the most frequently cited demonstration that failed mitochondrial clearance activates this sensor was retracted after publication, which a mechanistic review is obliged to state rather than absorb silently by citing something else.

1.2 Why the microglial cell is the right place to look

The case for treating microglia as the site of integration is not rhetorical. It rests on human genetics. Common and rare variants that modify late-onset Alzheimer's disease risk are concentrated in genes expressed by myeloid cells, and AD risk alleles are specifically enriched in the active enhancers of monocytes, macrophages and microglia rather than in neuronal regulatory elements.¹ Rare coding variants in *TREM2*, *PLCG2* and *ABI3* implicate microglial innate immunity directly.² The largest genome-wide association study of the disease and related dementias resolves seventy-five risk loci and finds microglial implication among its principal pathway signals.³ Whatever else Alzheimer's disease is, the inherited component of its risk is substantially a statement about myeloid cells.

Microglia are also the cell in which the six remaining elements can be co-located without special pleading. CLIC1 is enriched in human and murine microglia with minimal expression in other brain cells.⁴ Cathelicidin is expressed by microglia and astrocytes under inflammatory stimulation.⁵ Microglial lysosomal capacity is measurably compromised by disease-associated variants of *TREM2* and *SORL1*.⁶ Microglial mitochondrial state governs phagocytic competence.⁷ And cGAS–STING

upregulation in Alzheimer's models is microglia-dominant.⁸ The elements are in the same cell. Whether they are in the same pathway is the question.

1.3 What this review is and is not

This is a critical mechanistic review and experimental synthesis. It reports no new biological data. It considers primary literature indexed through 14 September 2026, with emphasis on studies that change the interpretation of causality, cell type, or direction of effect. Proposed integrations are labelled as proposals. Where a claim rests on a single study, that is stated. Where a claim rests on a study performed in skin, gut or vasculature and is being applied to brain, that is stated. Where no study exists, that is stated and the absence is treated as a finding.

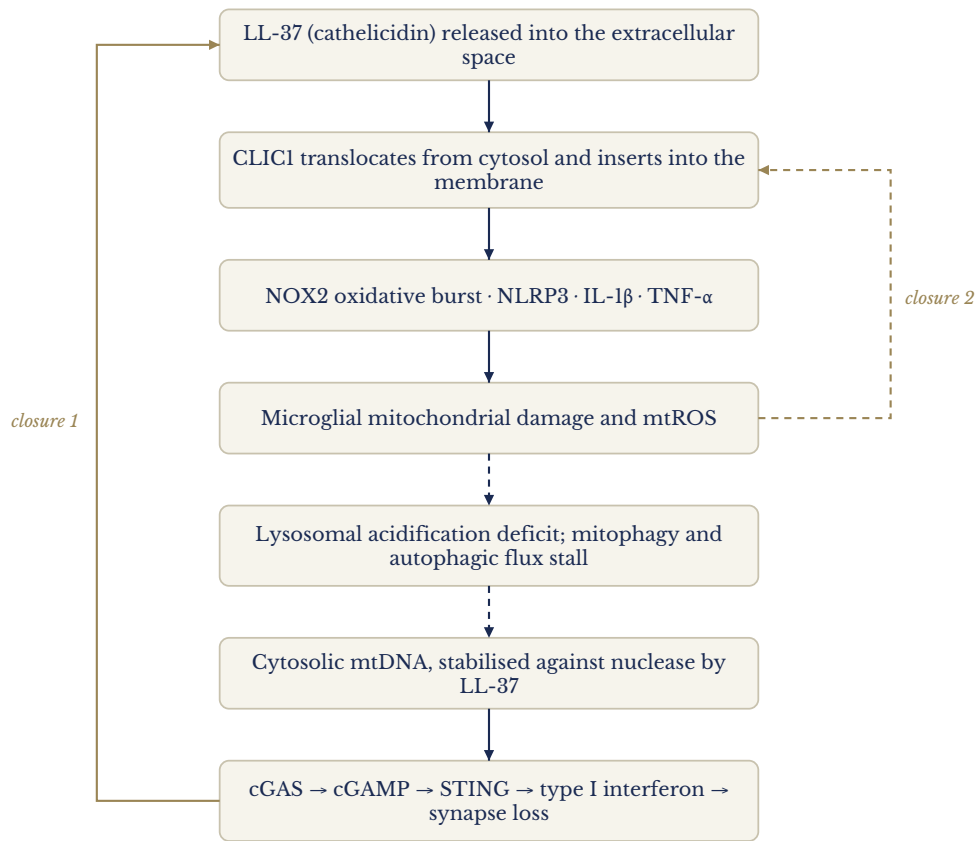
Three refusals govern the writing. The first: a mechanism demonstrated in a mouse is not thereby a mechanism in a human, and the specific ways in which rodent and primate microglia diverge — in complement, phagocytic and neurodegeneration-susceptibility gene modules — are precisely the modules this review depends on.⁹ The second: the cell-type attribution of an effect is a claim requiring evidence, and several effects historically assigned to microglia belong to perivascular macrophages.¹⁰ The third: where two bodies of work reach opposite conclusions, this review does not adjudicate by citing the more recent or the more prominent. It states the contradiction and identifies the experiment that would settle it.

1.4 Three questions that organize the argument

The first is **initiation**. What starts the circuit, and is the starting point inside the microglial cell or outside it? An extracellular peptide acting on a membrane-inserting protein is a different kind of event from an intracellular mitochondrial lesion, and the two imply different interventions. The evidence for both exists; their order does not.

The second is **maintenance**. A circuit that closes on itself does not require its initiating stimulus to persist. If interferon output raises transcription of the peptide that starts the sequence, and if mitochondrial oxidants promote insertion of the protein that transduces it, the circuit can be self-sustaining after the original insult resolves. This is the property that would make the arrangement worth calling a mechanism, and the property that is least tested.

The third is **termination**. Lysosomes degrade activated STING as well as its ligands, so a degradative deficit has two opposite-signed effects on one pathway: it raises the supply of cytosolic DNA and slows removal of the activated sensor. A therapy directed at either arm alone may move the wrong one. The concern is not hypothetical: the intervention most often proposed for the degradative deficit — restoring lysosomal acidification — has been shown in a non-neural system to be a requirement for, rather than a protection against, the lysosomal membrane damage that drives inflammasome activation.¹¹



Closure 1: interferon and infection raise CAMP transcription. Closure 2: mitochondrial ROS oxidises CLIC1 and promotes its insertion.

Dashed segments have not been tested in microglia or in brain tissue.

Figure 1. Solid arrows mark links with direct experimental support in some system; dashed arrows mark links inferred across tissue or cell type and not yet tested in microglia or in brain. The two gold returns are the closures that make this a circuit rather than a cascade. No step in this diagram has been demonstrated end to end in Alzheimer's disease tissue. Sources: ¹², ⁴⁴, ¹⁵⁰, ⁵⁶.

1.5 A note on how the elements were originally assembled

The seven terms treated here were set down together as a working note before the literature was surveyed, in the order microglia, mitochondrial DNA, LL-37, failed lysosome, CLIC1, Alzheimer's disease, cGAS–STING, mitophagy. That provenance is worth stating because it affects how the result should be read. The arrangement that follows was not derived from the note; it was tested against the literature, and two of the elements — CLIC1 and LL-37 — turned out to be joined by a published finding that the note did not assert and that most of the Alzheimer's literature has not absorbed.¹² The remaining connections were either found, found only in another tissue, or found to be absent. All three outcomes are reported.

2. How the Evidence Is Graded

2.1 The grading scale

Every proposed link in this review is assigned one of five levels. The scale measures the distance between what was actually observed and what the circuit requires. It does not measure study quality, and a Tier 3 finding may be technically superior to a Tier 1 one.

Tier 1 — Direct human disease evidence. The relationship has been observed in human Alzheimer's disease tissue, in human cells carrying a disease-causing or risk-modifying genotype, or in a human clinical cohort with disease endpoints.

Tier 2 — Disease-model evidence. The relationship has been demonstrated experimentally in an animal model of Alzheimer's disease or tauopathy, or in human cells under a manipulation designed to reproduce the disease state.

Tier 3 — Cross-system inference. The relationship has been demonstrated experimentally, but in a different cell type, tissue or disease. Transfer to microglia or to brain is an assumption of the model, not an observation.

Tier 4 — Untested prediction. The relationship is consistent with independently established components, and each component has been observed, but the relationship itself has not been measured anywhere in the form the circuit requires.

Tier 0 — Null. The relationship was searched for directly and no primary literature connects the two elements in any system. Tier 0 is a claim about the indexed literature, not about biology; it means no one has looked, or no one has published.

2.2 The inventory of elements

Table 1 — The seven elements, their identity in this circuit, and what is established about each independently of the others.

Element	Identity in the circuit	Established independently	Principal uncertainty
Microglia	The cell in which the circuit runs	Yolk-sac-derived, self-renewing, transcriptionally distinct; the cell type most enriched for AD risk alleles	Human and mouse microglial states diverge in exactly the modules used here
LL-37 / CAMP	Cationic initiator; also a DNA-handling cofactor	Single human cathelicidin gene; processed extracellularly by proteinase 3; binds and condenses DNA	Never measured in human AD brain; transport into brain never demonstrated
CLIC1	Metamorphic transducer at the membrane	Soluble GST-fold monomer converts to inserted oligomer under oxidation and low pH; microglia-enriched	Whether its microglial function is ionic at all is actively contested
Lysosome	Degradative terminus for both arms	Acidification requires presenilin-1-dependent v-ATPase delivery; fails early in AD models and human tissue	Whether microglial, as opposed to neuronal, acidification fails in AD is largely unmeasured
Mitophagy	The quality-control route that the lysosome completes	PINK1/ubiquitin-dependent and receptor-dependent routes; impaired markers in AD hippocampus	No flux measurement exists in human brain; basal route may not need PINK1
Mitochondrial DNA	The cargo and the ligand	Escapes by macropore, VDAC oligomer and permeability transition; a bona fide agonist of cGAS	Fluid measurements in AD run in the direction opposite to a leakage model
cGAS–STING	The sensor and amplifier	Structurally defined; activated in AD models and in human AD cortex; drives type I interferon	Also performs homeostatic autophagy and lysosomal functions that inhibition would remove

2.3 The inventory of links

Fourteen links are required for the circuit as drawn. They are listed here with their grades and are argued individually in Chapter 8. The pattern in this table is the substantive result of the review, and it is not a favourable one: the arms are well supported at their ends and weak at the joint.

Table 2 — The fourteen links, graded. E7 is the load-bearing weakness; E8 has lost its anchor citation to retraction.

#	Link	Tier	Strongest system in which it has been shown
E1	LL-37 → CLIC1 membrane insertion	2	Mouse and monkey in vivo; one laboratory, unreplicated
E2	CLIC1 → microglial NOX2 oxidative burst	3	Neonatal rat primary microglia; mechanism contested
E3	CLIC1 → NLRP3 assembly and IL-1 β release	2	Adult mouse microglia; mouse bone-marrow macrophages

#	Link	Tier	Strongest system in which it has been shown
E4	CLIC1 → phagosomal acidification and proteolysis	3	Mouse peritoneal macrophages and dendritic cells
E5	CLIC1 → lysosomal acidification	0	Never tested in any system
E6	Microglial oxidative load → mitochondrial damage	3	Macrophages; general cell biology
E7	Lysosomal acidification deficit → stalled mitophagy in AD	4	Asserted in review; never directly measured
E8	Stalled mitophagy → cytosolic mtDNA	3	Aged macrophages, arthritis, retina; anchor paper retracted
E9	Cytosolic mtDNA → cGAS–STING activation in AD	1	Human AD brain; tauopathy and amyloid mice
E10	cGAS–STING → type I interferon → synapse loss	2	Multiple murine amyloid models, cell-type resolved
E11	Interferon and infection → CAMP transcription	3	Human macrophages and glia; regulation is primate-specific
E12	Mitochondrial ROS → CLIC1 oxidation and insertion	3	Mouse macrophages; ordering established for inflammasome
E13	LL-37 carries DNA and cGAMP into the cytosol → STING	3	Human primary monocytes; mast cells; skin macrophages
E14	LL-37-mtDNA complexes resist nuclease and escape autophagy	3	Human atherosclerotic plasma and plaque; septic lung

2.4 What would count as refutation

A model that cannot fail is not worth stating. Four observations would each falsify the circuit as drawn, and each is obtainable with existing methods.

If LL-37 or its precursor cannot be detected in human Alzheimer's brain tissue above the level found in age-matched control tissue, the initiating arm is removed and the circuit reduces to the already-known mitochondrial DNA–interferon axis. If CLIC1 is present in human Alzheimer's microglia only in its soluble form, with no increase in the membrane-inserted species relative to control tissue, the transducing step is removed. If microglial mitophagic flux in an Alzheimer's model is normal or increased at the stage when cytosolic mitochondrial DNA rises, then the degradative bottleneck is not the source of the ligand, and the circuit's central joint fails. And if deleting CLIC1 selectively in microglia leaves cytosolic mitochondrial DNA and interferon-stimulated gene induction unchanged in a model carrying both amyloid and tau pathology, then the peptide–channel arm is not necessary for the sensing arm, whatever else it does.

These are the experiments described in Chapter 13. They are ordered so that the first of them, which is the cheapest, is also the one most likely to end the enquiry.

3. The Cell: What a Microglial State Is and Is Not

3.1 Surveillance as the baseline

Microglia arise from yolk-sac primitive macrophages before definitive haematopoiesis and maintain themselves in the brain thereafter as a self-renewing population dependent on CSF1R.¹³ Their resting condition is not rest. Two-photon imaging of intact cortex showed that processes of so-called resting microglia survey their territory continuously and reorganise within minutes.¹⁴ Their transcriptional identity is a distinct, TGF- β -dependent signature not shared with other myeloid cells and not retained by cell lines.¹⁵

That last point is a methodological constraint on most of the literature used in this review. Human microglia isolated from surgically resected brain lose their microglia-specific genes rapidly once removed from the tissue environment, and many of the genes lost are the ones altered in neurodegenerative disease.¹⁶ A large part of the CLIC1 evidence base, and essentially all of the older microglial ion-channel electrophysiology, comes from cultured neonatal rodent cells. Those cells are not adult human microglia in a diseased brain, and the difference is not a minor one.

3.2 Disease-associated states and the limits of naming them

Single-cell analysis of an amyloid model identified a disease-associated microglial programme containing phagocytosed amyloid, activated in two steps of which the second requires TREM2.¹⁷ An independently derived framework described an APOE-dependent switch from the homeostatic to a neurodegenerative phenotype triggered by phagocytosis of apoptotic neurons.¹⁸ A time-course study across sexes and genotypes separated an activated response population from an interferon response population, and found that *ApoE* regulates the first but not the second.¹⁹ The largest human atlas to date resolves twelve microglial states across hundreds of subjects and infers the transcription-factor transitions between them.²⁰

These frameworks are not interchangeable and their valences differ. The field's own consensus statement warns that state labels silently import assumed functions, and that a transcriptional category is not a demonstrated behaviour.²¹ The warning applies directly here: the circuit proposed in this review predicts a particular microglial behaviour — an inserted channel, a failed lysosome, a leaking mitochondrion — and none of those is read off a transcriptional state.

The translation problem is sharper still. Comparative single-cell analysis across ten species found that rodent and primate microglia diverge specifically in complement, phagocytic and neurodegeneration-susceptibility modules.⁹ A direct human–mouse comparison confirmed TREM2-dependent disease-associated microglia in a mouse model while finding markedly different glial phenotypes in human Alzheimer's tissue, closer to an IRF8-driven reactive profile.²² Human stem-cell-derived microglia transplanted into mouse brain respond to oligomeric amyloid divergently from mouse microglia in the same tissue.²³ And in one of the few studies using live-sorted human microglia, the subset associated with Alzheimer's disease was *reduced* in frequency in disease tissue rather than gained.²⁴

3.3 The case that microglial failure, not microglial activation, is the lesion

A consistent line of human neuropathology argues the opposite of the neuroinflammation framing. High-resolution analysis of microglia adjacent to tau-positive structures in human Alzheimer's brain finds dystrophic rather than activated cells, and concludes that microglial senescence precedes neurofibrillary degeneration.²⁵ The same programme reports that microglial dystrophy correlates in situ with neurofibrillary degeneration, and interprets a chronic and futile reaction to insoluble amyloid as exhausting the cell.²⁶ A partial reconciliation exists: early sustained microglial proliferation drives replicative senescence, and preventing that proliferation blocks both senescence and the disease-associated programme, which suggests the two literatures may be describing successive phases of one trajectory.²⁷

This matters for the present circuit in a specific way. Everything proposed here — channel insertion, oxidative burst, inflammasome assembly, degradative overload — describes a cell working hard and working badly. That is compatible with both framings. It is not compatible with a picture in which microglia are simply inert.

3.4 Priming, and why a peripheral peptide is a plausible input

Microglia in a chronically altered brain respond to a peripheral inflammatory stimulus far more strongly than microglia in a normal one. The paradigm was established in prion-diseased mice, where peripheral endotoxin produced exaggerated sickness behaviour absent in controls,²⁸ and extended to acute working-memory deficits in the primed but not the normal brain. In humans, a prospective cohort of three hundred community-dwelling patients with Alzheimer's disease found that acute systemic inflammatory events accompanied by raised TNF- α were associated with long-term cognitive decline.²⁹

Priming is what makes an infection-inducible peptide a credible input to a neurodegenerative circuit rather than an exotic one. It supplies the intermittent, systemically triggered signal that a self-closing loop would need in order to be initiated repeatedly over years. It does not, by itself, demonstrate that the peptide reaches the brain — an omission taken up in Chapter 5.

3.5 The disagreement that governs interpretation of every intervention

Removing microglia from an amyloid model prevents neuronal loss without changing amyloid pathology.³⁰ Removing them earlier and for longer prevents parenchymal plaques from forming at all, redirecting amyloid into a vascular distribution.³¹ Removing them in a different model for four weeks changes neither plaque formation nor maintenance nor amyloid-associated neuritic dystrophy.³² Removing them from healthy adult visual cortex increases excitatory and inhibitory connectivity and raises neuronal activity, all reversibly.³³

These results are not reconcilable by choosing the best study. Timing, duration and degree of depletion drive the outcome, and any mechanistic claim about microglia in Alzheimer's disease that does not specify disease stage is underdetermined. The same discipline is required of the circuit proposed here, and Chapter 13 accordingly specifies stage for every proposed intervention.

4. CLIC1: A Protein Without a Fixed Structure

4.1 Why an unusual protein deserves a full treatment

CLIC1 is the least familiar element in this circuit and the one whose properties do most of the mechanistic work. It was cloned from activated myelomonocytic cells as a nuclear chloride channel and named NCC27.³⁴ Its crystal structure at 1.4 Å resolution then showed something the name did not anticipate: a soluble monomer with a glutathione-transferase fold carrying a glutaredoxin-like redox-active site occupied by glutathione.³⁵ It is, structurally, a soluble enzyme-like protein that also becomes an integral membrane protein.

That conversion is the whole of its interest. Oxidation drives a reversible transition from monomer to a non-covalent dimer through an intramolecular Cys24–Cys59 disulfide, a result that founded the description of CLIC1 as a metamorphic protein.³⁶ Oxidation promotes insertion into membranes.³⁷ On interacting with a bilayer the protein extends a transmembrane segment and oligomerises, which it must do because each monomer contributes only one membrane-spanning helix and a pore requires more than one.³⁸ Insertion is cholesterol-dependent through a conserved GXXXG motif in that helix.³⁹ Protonation of specific histidines stabilises the intermediate state at acidic pH.⁴⁰ A separate two-step mechanism identifies intracellular zinc release as an activation trigger distinct from oxidation and pH.⁴¹ Channel behaviour in planar bilayers is set by the redox potential on the luminal face and requires a particular lipid composition for assembly, so insertion and conduction are separable events.⁴²

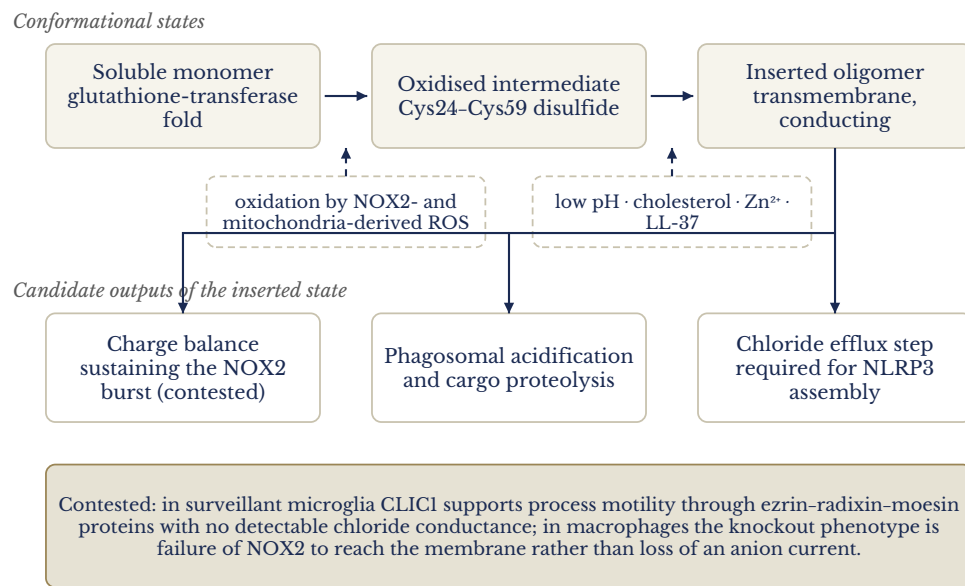


Figure 2. *CLIC1* has no fixed structure. The same polypeptide is a soluble glutathione-transferase-fold monomer, an oxidised intermediate, and an inserted membrane oligomer. The insertion triggers are established *in vitro*. The three outputs are separately evidenced and partly contested: the charge-balance role is challenged by the finding that surveillant microglia show no *CLIC1* chloride conductance at all. Sources: ^{36, 37, 51, 4, 41}.

The functional consequence is that *CLIC1* is sensor and effector in one molecule: it reads the oxidative and acidic state of its surroundings and converts that reading into a change in membrane composition. For a circuit in which oxidative burst and lysosomal acidification are both variables, this is the property that matters.

4.2 What CLIC1 does in microglia, and the evidence that it does something else

The original microglial result is now more than twenty years old. Amyloid- β stimulation of neonatal rat microglia raised CLIC1 protein and produced a functional plasma-membrane chloride conductance barely detectable in quiescent cells, coincident with reactive nitrogen intermediate and TNF- α production; blocking the conductance with IAA-94 prevented apoptosis in co-cultured neurons, and small interfering RNA against CLIC1 prevented amyloid-induced TNF- α release.⁴³ A follow-up established the translocation directly: amyloid promotes acute movement of CLIC1 from cytosol to plasma membrane, and both the chloride conductance and the reactive oxygen species generation are prevented by pharmacological inhibition, by anion substitution, by an anti-CLIC1 antibody and by knockdown. Crucially, CLIC1 activation was itself found to depend on oxidation by NADPH-oxidase-derived species, which the authors proposed as a feedforward mechanism sustaining the burst.⁴⁴ A third study dissociated the toxic from the protective arm: blocking CLIC1 preserved, and modestly increased, amyloid phagocytosis and degradation while suppressing inducible nitric oxide synthase induction.⁴⁵ The finding was extended from the truncated peptide to full-length fibrillogenic amyloid- β 1-42, with a useful negative control: neurotoxicity driven by lipopolysaccharide with interferon- γ was insensitive to the same blockers, indicating selectivity in which stimuli recruit the channel.⁴⁶ Independent proteomic work found CLIC1 among the cysteine-redox-modified proteins in amyloid-challenged microglia, tying the oxidation to the transition in the relevant cell.⁴⁷ The charge-balance interpretation — that the anion current neutralises charge extruded by an active NADPH oxidase — was articulated as a general model of CLIC1 as both second messenger and executor of oxidative stress.⁴⁸

A 2025 study substantially revises this picture. CLIC1 was confirmed as highly enriched in human and murine microglia with minimal expression elsewhere in brain, and blockade or genetic deletion was found to attenuate surveillance by reducing ramification and motility without affecting chemotaxis, a phenotype reproduced in xenografted human microglia and in human brain tissue. The mechanism, however, runs through interactions with the actin-binding ezrin, radixin and moesin proteins, and patch-clamp recording found no chloride conductance in surveillant microglia at all. The authors conclude that CLIC1's function in these cells is chloride-independent and unlikely to reflect ion channel activity. The same study found that after ATP-evoked activation, CLIC1 blockade strongly suppresses NLRP3-dependent interleukin-1 β release.⁴

This correction should not be smoothed over. It preserves the two results this circuit needs — that CLIC1 is microglia-enriched and that its blockade suppresses inflammasome output — while removing the mechanism by which those results were explained for two decades. A parallel correction exists outside brain: in CLIC1-null mice the failed macrophage oxidative burst is attributable to failure of NADPH oxidase to redistribute to the plasma membrane rather than to loss of an anion conductance.⁴⁹ Two independent systems point to CLIC1 acting on membrane organisation and trafficking rather than on ion flux.

The revision is also not unopposed. A 2025 study in an amyloid model reports a circular RNA encoded by *APP* acting through a microRNA to raise both CLIC1 expression and chloride-channel activity in microglia, with knockdown of either improving pathology and cognition — a functional chloride readout obtained in a disease model rather than in culture.⁵⁰

The revision changes the mechanism but not the position of the node. A protein that must insert into a membrane before it can organise that membrane's cytoskeletal and oxidase machinery remains a redox-gated switch between the extracellular environment and the microglial inflammatory output. What changes is the readout: an experiment testing this circuit should measure the inserted species and its consequences, not a chloride current.

4.3 CLIC1 at the phagosome, and the inference this review draws from it

The finding that does most to connect CLIC1 to the degradative arm was made in macrophages. On phagocytosis of opsonised zymosan, CLIC1 translocates onto the phagosomal membrane. Macrophages from CLIC1-null mice show a defect in phagosomal acidification measured in live cells with a pH-sensitive fluorophore. The defect is not explained by failed fusion — phagosome–lysosome fusion was unimpaired — and it is accompanied by impaired phagosomal proteolytic capacity and reduced reactive oxygen species production. CLIC1-null mice were protected from serum-transfer arthritis.⁵¹ The same mechanism was reproduced in dendritic cells, where CLIC1 loss or pharmacological blockade impaired phagosomal acidification and proteolysis, reduced antigen processing and presentation, and reduced experimental autoimmune encephalomyelitis.⁵²

The thermodynamic rationale for a counterion requirement is not in doubt. Proton pumping by the v-ATPase separates charge and builds a membrane potential that limits further acidification unless a counterion conductance dissipates it.⁵³ Endosomal and lysosomal CLC transporters are the canonical candidates for that role,⁵⁴ and their biophysics is well characterised.⁵⁵ CLIC1 is not one of them and has never been shown to serve that function in a lysosome.

This produces the chapter's most important qualification. CLIC1's acidification role is demonstrated at the **phagosome**, in **macrophages and dendritic cells**, and its extension to the **lysosome** in **microglia** is an inference. A targeted search returns no study testing CLIC1 as a lysosomal counterion conductance in any system. The link is graded Tier 0 and is one of two places where this circuit is held together by an assumption rather than an observation.

4.4 CLIC1 and the inflammasome

The connection to NLRP3 is better supported and is independently ordered. In mouse bone-marrow macrophages, chloride efflux through CLIC proteins is an essential and proximal upstream event for NLRP3 activation, and the established sequence runs: agonist, potassium efflux, mitochondrial damage and mitochondrial reactive oxygen species, CLIC translocation to the plasma membrane, chloride efflux, NEK7–NLRP3 interaction, ASC assembly, caspase-1, interleukin-1 β .⁵⁶ CLIC1 and CLIC4 also act at the priming step, with knockdown of either impairing interleukin-1 β transcription as well as speck formation and mature cytokine secretion.⁵⁷ Extracellular acidosis lowers intracellular pH in macrophages and drives agonist-induced CLIC1 translocation to the plasma membrane, amplifying caspase-1 processing — a direct pH-to-channel-to-inflammasome link.⁵⁸ And in microglia specifically, CLIC1 blockade strongly suppresses NLRP3-dependent interleukin-1 β release after ATP.⁴

The ordering in the macrophage work deserves emphasis because it supplies one of the two closures of the circuit. Mitochondrial reactive oxygen species are placed *upstream* of CLIC translocation. Combined with the finding that oxidation is what drives CLIC1 insertion,³⁶³⁷ and with the microglial finding that CLIC1 activation depends on oxidase-derived species,⁴⁴ this makes mitochondrial damage a cause of channel insertion and not only a consequence of it.

4.5 What is known about CLIC1 in human Alzheimer's disease

Very little, and the little that exists is peripheral. Confocal and electrophysiological analysis demonstrated transmembrane CLIC1 in peripheral blood mononuclear cells from patients with neurodegeneration, establishing that the inserted species can be detected in patient cells.⁵⁹ The distinction between total CLIC1 and the membrane-inserted form has been argued to be the pathology-specific one, with the inserted species as the biomarker and therapeutic target.⁶⁰ In blood transcriptomic data from a large Alzheimer's cohort, CLIC1 was the only probe surviving multiple-testing correction across clinical dementia rating groups, while being insufficient alone for classification.⁶¹ Re-analysis of a prefrontal cortex expression dataset places CLIC1 among oxidative-stress genes distinguishing Alzheimer's brain, with the direction validated in mouse temporal cortex.⁶² An independent analysis identifies CLIC1 as a shared immune-dysregulation gene across Alzheimer's and sepsis-associated cognitive impairment, upregulated in model animals and in cytokine-stimulated human cerebral endothelial cells.⁶³

No study has performed immunohistochemistry or proteomics for CLIC1 on human Alzheimer's brain tissue, and no study has distinguished the soluble from the membrane-inserted species in that tissue. The only human brain-tissue result in the literature is the demonstration of microglial enrichment in the 2025 study, which was not performed in an Alzheimer's context.⁴ This absence is the first item in the experimental programme of Chapter 13 for the simple reason that it is cheap, decisive, and has not been done.

4.6 Pharmacology, and a knockout phenotype worth noticing

CLIC1 is druggable and one of its inhibitors is already a widely used medicine. Metformin is a direct CLIC1 inhibitor; it reproduces block of the CLIC1-dependent current, and substituting arginine 29 abolishes its modulation.⁶⁴ Inhibition of CLIC1 is a biguanide class effect rather than a metformin idiosyncrasy.⁶⁵ Single-channel recording with nuclear magnetic resonance has since shown metformin binding the membrane-inserted form directly through Arg29, with CRISPR knockout or Arg29 mutation abolishing the drug's effect and efficacy achieved at nanomolar tumour concentrations in vivo.⁶⁶ A distinct chemotype blocks the transition rather than the pore: a cryptic-site ligand oligomerises the soluble protein and reduces membrane expression.⁶⁷ The classical tool compounds remain IAA-94 and niflumic acid.⁴³

The knockout phenotype is unusually favourable for a therapeutic target. CLIC1-null mice are viable, fertile and grossly normal, with a mild platelet defect as the only baseline abnormality,⁶⁸ a picture confirmed independently in a separate line where global knockouts were overtly normal yet tumour growth was suppressed.⁶⁹ Against that near-silent baseline, the same animals are protected in serum-transfer arthritis,⁵¹ in experimental autoimmune encephalomyelitis,⁵² in toxic organ injury,⁴⁹ and — the result that brings this chapter into contact with the next — in peptide-driven Alzheimer's-like pathology.¹²

A constitutive activity that is dispensable in health and required under challenge is the profile of a good target. It is also, unavoidably, the profile of a protein whose relevance to a human disease has not yet been demonstrated in human diseased tissue.

5. LL-37: A Host-Defence Peptide That Handles DNA

5.1 One gene, one peptide, several molecules

Humans carry a single cathelicidin gene. Its product hCAP-18 is stored in neutrophil specific granules and cleaved extracellularly by proteinase 3 to the mature 37-residue peptide LL-37.⁷⁰ The peptide is chemotactic for neutrophils, monocytes and T cells through the formyl peptide receptor FPR2,⁷¹ signals through P2X7,⁷² and has an intracellular binding partner, GAPDH, identified in human monocytes — which matters because the mechanisms this review needs require the peptide to act inside the cell and not only at its surface.⁷³

Transcription of the gene is inducible in ways that make the peptide a plausible link between systemic events and brain. It is a direct target of the vitamin D receptor,⁷⁴ and Toll-like receptor activation of human macrophages upregulates the receptor and the activating hydroxylase, producing cathelicidin induction that fails when circulating vitamin D is low.⁷⁵ This is a genuinely important constraint on modelling: the vitamin-D response element in the human gene arose from a primate-specific Alu element, and non-primate mammals lack this regulation entirely.⁷⁶ Any mouse experiment using the murine orthologue CRAMP therefore does not model the human induction pathway, only the peptide's downstream actions.

The phrase "LL-37" also conceals real heterogeneity. Citrullination abolishes the peptide's ability to deliver DNA and to activate plasmacytoid dendritic cells, while carbamylation does not.⁷⁷ Neutrophil-released peptide, which is N-terminally modified, fails to induce autophagy in human macrophages, whereas macrophage-derived native peptide does.⁷⁸ Host-cell toxicity depends on aggregation state. Post-translational modification and cell of origin should therefore be specified whenever the molecule is invoked, and rarely are.

5.2 The property that makes this peptide unusual: it changes what DNA does

The founding observation is that plasmacytoid dendritic cells sense self-DNA when it is coupled to LL-37. The peptide converts otherwise inert self-DNA into a potent interferon trigger by forming condensed aggregates retained in early endosomes, where they engage TLR9.⁷⁹ The complexes are produced physiologically as neutrophil extracellular traps.⁸⁰ The amplification is quantitatively large: LL-37 lowers the threshold for CpG sensing by twenty- to thirtyfold and works in serum and whole blood.⁸¹

More directly relevant here, the peptide also delivers DNA past the endosome. LL-37 transports self-DNA into human primary monocytes and produces type I interferon in a Toll-like-receptor-independent manner; induction occurs with B-form double-stranded DNA regardless of sequence, CpG content or methylation, and **requires STING and TBK1** — that is, it requires the cytosolic sensing pathway.⁸² A second transport function has since been described: LL-37 binds and carries extracellular cGAMP into cells to activate STING, meaning the peptide can amplify the pathway at the messenger level as well as the ligand level.⁸³

The peptide's relationship to mitochondrial DNA specifically is the most direct bridge in this review. LL-37-mitochondrial DNA complexes are elevated in atherosclerotic plasma and plaque, are **resistant to DNase II degradation**, and **escape autophagic recognition**; the murine equivalent worsens lesions and an antibody against the complex rescues.⁸⁴ The same resistance mechanism was reproduced in a second organ system, where the peptide conferred resistance to DNase-II-mediated mitochondrial DNA degradation and blocked the resulting autophagy.⁸⁵ Adding LL-37 to mitochondrial DNA CpG islands induces spontaneous interferon- α production from human plasmacytoid dendritic cells,⁸⁶ and oxidised mitochondrial DNA — the species most relevant in a

high-oxidative-load cell — is a stronger stimulus still.⁸⁷ In human patients, plasma LL-37 correlates positively with circulating extracellular DNA.⁸⁸

A single recent study assembles most of the circuit in a non-neural cell: LL-37 causes reactive-oxygen-driven mitochondrial damage and permeability transition pore opening, leading to cytosolic mitochondrial DNA leakage and cGAS/STING activation, rescued by antioxidant, by pore blockade and by STING degradation.⁸⁹ A second, independent line reports LL-37-induced STING-mediated inflammation in skin macrophages with elevated nuclear DNA fragmentation in lesions.⁹⁰ Both are skin; neither is brain.

The direction is not uniform. In psoriatic keratinocytes LL-37 **neutralises** cytosolic DNA and **blocks** AIM2 inflammasome activation.⁹¹ The peptide is pro-inflammatory through TLR9 and STING and anti-inflammatory through AIM2, with compartment setting the sign. Whether that split exists in microglia is unknown.

5.3 Cathelicidin in brain

Cathelicidin is present in the central nervous system. The rat orthologue was detected in brain extract with bactericidal activity,⁹² and LL-37 is present in human cerebrospinal fluid in bacterial meningitis but not in control cerebrospinal fluid, with rat astrocytes and microglia expressing and secreting the orthologue on bacterial stimulation.⁹³ The most important prior for the present argument reports that hCAP18/LL-37 is expressed across human organs with among the highest basal levels in gastrointestinal tract and brain, that interferon- γ raises the peptide in human astrocytes and lipopolysaccharide with interferon- γ raises it in human microglia, and that the peptide drives glial neuroinflammation.⁵ Amyloid- β itself induces the murine orthologue in microglia through ERK, p38 and NF- κ B.⁹⁴

Taken together these supply closure 1 of the circuit: an inflammatory or interferon signal, or amyloid itself, raises transcription of the peptide that initiates the sequence. The evidence is Tier 3 — human glia in culture and rodent cells — and the primate-specific nature of the vitamin-D arm makes murine confirmation of that particular input impossible in principle.

The direction of cathelicidin's effect in brain is, however, genuinely unsettled, and the disagreement is not between weak and strong studies. Cathelicidin-deficient mice show higher mortality and bacterial burden in meningitis with upregulated pro-inflammatory genes.⁹⁵ Intracerebroventricular infusion of the peptide reduces mortality, glial activation and inflammation in pneumococcal meningitis.⁹⁶ Exogenous peptide inhibits endotoxin-driven glial cytokine production in vitro and in vivo.⁹⁷ Against these, cathelicidin is elevated in experimental autoimmune encephalomyelitis spinal cord and drives astrocyte-microglia neuroinflammation, with corroborating post-mortem multiple sclerosis tissue,⁹⁸ and cathelicidin released from neutrophils, microglia and astrocytes drives severe disease in the same model through Th17 differentiation.⁹⁹ Outside the nervous system the split repeats: endogenous cathelicidin worsens myocardial ischaemia-reperfusion injury through TLR4 and the P2X7-NLRP3 axis,¹⁰⁰ while it promotes repair after acetaminophen liver injury.¹⁰¹

Acute infection and chronic sterile inflammation appear to give opposite answers. A circuit proposed for a chronic neurodegenerative disease should be read against the chronic results, and should be stated as being in tension with the acute ones.

5.4 The peptide and amyloid

In vitro, LL-37 binds amyloid- β 42 specifically and **inhibits** fibril assembly, altering fibril morphology.¹⁰² Detailed biophysics shows the peptide and its truncated fragments forming hetero-oligomers and nanoclusters with amyloid- β 40, with the strongest fibrillation inhibitors forming more hetero-oligomers and smaller off-pathway clusters.¹⁰³ The peptide is a nanomolar inhibitor of islet amyloid polypeptide self-assembly, indicating a general amyloid-modulating capacity rather than an amyloid- β idiosyncrasy.¹⁰⁴ And the peptide is itself amyloidogenic: its active core self-assembles into a fibril of densely packed helices, and that self-assembly is required for its antibacterial activity.¹⁰⁵

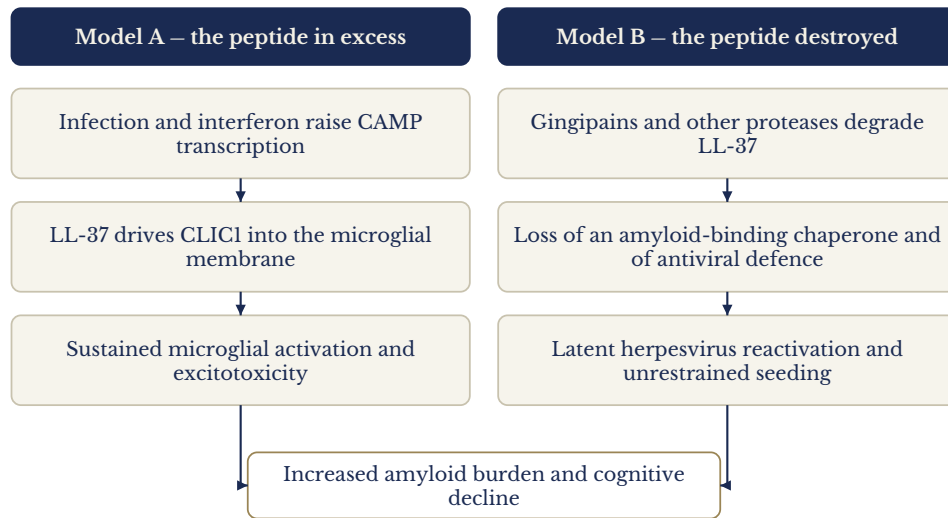
This is the point at which LL-37 intersects the antimicrobial protection hypothesis of Alzheimer's disease. Amyloid- β was shown to be an antimicrobial peptide with potency benchmarked explicitly against LL-37,¹⁰⁶ to protect against microbial infection in vivo with oligomerisation required for the activity,¹⁰⁷ and the hypothesis was formalised as a proposal that amyloid deposition is an innate-immune act whose accompanying inflammation propagates degeneration.¹⁰⁸ On that reading, the brain contains at least two amyloidogenic host-defence peptides that bind each other, and the disease is a disorder of an immune effector system rather than of a waste product.

5.5 The peptide in Alzheimer's disease, and the contradiction at the centre of this review

The anchor result is a 2022 study reporting that LL-37 promotes CLIC1 membrane translocation and integration and activates it, causing microglial hyperactivation, neuroinflammation and excitotoxicity; that in mouse and monkey models the peptide produced elevated amyloid- β , increased neurofibrillary tangles, neuronal death, brain atrophy, ventricular enlargement and impaired synaptic plasticity and cognition; and that *Clic1* knockout or blockade of the peptide-channel interaction prevented these outcomes.¹² It is an unusually complete study, spanning rodent and non-human primate, with both genetic and interaction-level rescue.

It is also, four years later, unreplicated: a targeted search returns no independent reproduction, commentary or critique. The only human follow-up is a two-year study of 357 older adults with memory complaints, and its result is mixed in a way that requires care. Serum LL-37 showed no association with the continuous rate of change in Mini-Mental State Examination score, while high LL-37 was associated with the odds of a three-point drop and with steeper rises in neurofilament light and phosphorylated tau 181.¹⁰⁹ The headline-supportive findings are the secondary ones.

Against this stands an equally serious programme arguing the opposite sign. On that account, Alzheimer's disease arises in part from the **loss** of LL-37 — degraded along with other innate immune proteins by *Porphyromonas gingivalis* gingipains — removing a peptide that binds amyloid- β and restrains amyloid formation, and permitting herpesvirus reactivation and consequent amyloid production.¹¹⁰ A recent review frames amyloid- β and LL-37 as two sides of one coin, both protective acutely and injurious chronically.¹¹¹



Both routes terminate in the same observable, so amyloid burden cannot adjudicate between them.

Figure 4. *The same peptide is placed at opposite ends of the same disease. Both models predict increased amyloid, so amyloid burden cannot discriminate them. The sign of a controlled perturbation can. Sources: ¹², ¹¹⁰, ¹⁰².*

These are not compatible as stated. One model requires more of the peptide to be worse; the other requires less of it to be worse. Both predict increased amyloid burden, so amyloid cannot discriminate them. Chapter 10 returns to this, proposes a biphasic reading, and specifies the experiment that would decide.

5.6 The hole in the initiating arm

One gap deserves to be stated plainly rather than buried in the grading table. There is no pharmacokinetic or transport study showing that peripheral LL-37 reaches brain parenchyma. The entire literature pairing the peptide with the blood–brain barrier consists of a handful of papers, none of which measures transport. There is also no measurement of LL-37 or its precursor in human Alzheimer's brain tissue, and no case–control measurement in Alzheimer's cerebrospinal fluid; the only demonstration of the peptide in human central nervous system fluid is in acute bacterial meningitis.⁹³

The circuit therefore has two possible initiating geometries, and the literature does not distinguish them. Either the peptide is made locally by glia under inflammatory or interferon drive — for which there is direct human-cell evidence⁵ — or it arrives from the periphery, for which there is none. The local-production geometry is the more defensible and is the one assumed hereafter. It also has the advantage of being the geometry that closure 1 requires.

6. The Degradative Arm: Lysosomal Failure and Stalled Mitophagy

6.1 Acidification is a defended set-point, not a maximum

The lysosome does not simply become as acidic as it can. Proton pumping by the v-ATPase separates charge and generates a membrane potential that opposes further pumping, so acidification requires a counterion conductance to proceed.⁵³ Endosomal and lysosomal CLC proteins provide part of that function, and disruption of individual members impairs compartment acidification.^{54,55} ClC-7 operates in an obligate complex with its β -subunit Ostml.¹¹²

Two findings prevent a naive reading in which more acid is better. Lysosomal pH is actively limited by a proton leak: TMEM175 is a proton-activated, proton-selective channel that terminates over-acidification, which reframes lysosomal pH as a regulated set-point.¹¹³ And gain-of-function variants of ClC-7 that accelerate voltage-dependent activation impair autophagic clearance as effectively as loss-of-function variants do.¹¹⁴ Deviation in either direction is pathological. A therapeutic proposal to "restore acidification" is therefore a proposal to restore a set-point, not to lower a pH.

Lysosomal ion handling also reaches back to mitochondria. Loss of the lysosomal potassium channel TMEM175 impairs mitochondrial function and increases α -synuclein aggregation,¹¹⁵ and lysosomal calcium release through TRPML1 activates calcineurin and drives TFEB nuclear translocation independently of mTOR,¹¹⁶ with mitochondrial reactive oxygen species acting directly on that channel.¹¹⁷ The two organelles are coupled in both directions before any disease is invoked.

6.2 Lysosomal acidification fails early in Alzheimer's disease

The foundational result is that presenilin-1 holoprotein is required for N-glycosylation and delivery of the v-ATPase V0a1 subunit to the lysosome, and that presenilin-1 loss or familial Alzheimer's mutation selectively blocks autolysosome acidification and cathepsin activation.¹¹⁸ Independent genetic support came from *Drosophila*, where loss of V0a1 in photoreceptor neurons causes adult-onset degeneration and sensitises neurons to human amyloid- β and tau.¹¹⁹

The most consequential development is the description of autolysosomal acidification failure preceding extracellular amyloid deposition. Autolysosome acidification and v-ATPase activity fall before plaques appear; amyloid- β and APP β -C-terminal fragments accumulate inside de-acidified autolysosomes; autophagic vacuoles pack into perikaryal rosettes; membrane permeabilisation and cathepsin release then produce lysosomal cell death with microglial invasion. Individual neurons in this state are identified as the principal source of senile plaques, and the pattern is present in human Alzheimer's brain.¹²⁰ A parallel and partly independent route exists: APP C-terminal fragments at late-endosome-lysosome-endoplasmic-reticulum contact sites reduce lysosomal calcium refilling and drive cholesterol accretion, causing a graded endolysosomal collapse rescued by APP depletion.¹²¹ The current synthesis of the field treats autophagy-lysosome dysfunction as central and stage-specific across neurodegenerative diseases.¹²²

That the deficit is correctable in vivo is established: inhibition of glycogen synthase kinase-3 restores lysosomal acidification in an amyloid model.¹²³

Almost all of this work is neuronal. The microglial arm is thinner but real. Microglia carrying *TREM2* risk variants, and *Trem2*-null microglia in an amyloid model, accumulate autophagic vesicles because of defective mTOR signalling and an ATP shortfall, correctable by a dietary energetic substrate.¹²⁴ Human induced-pluripotent-stem-cell microglia carrying a *TREM2* loss-of-function

mutation show lysosomal and lipid-handling failure.⁶ Lipid-droplet-accumulating microglia in the aged mouse and human brain are phagocytosis-defective, reactive-oxygen-species-high and cytokine-secreting.¹²⁵ Microglia also digest plaques extracellularly by forming acidic lysosomal synapses and exocytosing lysosomal enzymes onto material too large to engulf, a mechanism that makes microglial acidification capacity directly relevant to amyloid handling.¹²⁶

6.3 Mitophagy and what "impaired" means

Mitophagy proceeds through PINK1-generated phospho-ubiquitin, which recruits the autophagy receptors NDP52 and optineurin upstream of LC3.¹²⁷ In Alzheimer's disease, mitophagy is reported impaired in patient hippocampus, in induced-pluripotent-stem-cell-derived neurons and in animal models; nicotinamide supplementation, urolithin A and actinonin reverse memory loss, reduce insoluble amyloid- β — notably through enhanced microglial phagocytosis of plaques — and abolish tau hyperphosphorylation.¹²⁸ Human post-mortem evidence shows parkin becoming insoluble and sequestered with intraneuronal amyloid- β alongside autophagosome accumulation.¹²⁹ The mechanistic framing holds that failure to remove damaged mitochondria produces a bioenergetic deficit that itself drives amyloid and tau, closing a feed-forward loop.¹³⁰¹³¹

Three complications need to be carried forward rather than set aside. First, basal mitophagy in neural tissue of high metabolic demand is unchanged in PINK1-null mice, which questions whether the PINK1–parkin axis is the route neurons actually use day to day.¹³² Second, in reporter mice examined across multiple organs, mitophagy in old animals is increased or unchanged rather than decreased, complicating the premise that mitophagy declines with age.¹³³ Third, lysosomes remove the mitochondrial inner membrane piecemeal through cytosolic vesicles at rest,¹³⁴ and mitochondria-derived vesicles deliver assembled complexes to lysosomes independently of canonical mitophagy.¹³⁵ Both sub-organellar routes converge on the lysosome and are invisible to conventional mitophagy reporters. An intact reporter signal does not exclude a quality-control failure, and the converse also holds.

Human data are thin in a specific way. There is no flux measurement in human brain. The statement that mitophagy is impaired in Alzheimer's brain rests on static marker measurements, and static markers cannot distinguish reduced initiation from failed completion. That distinction is precisely the one this circuit depends on.

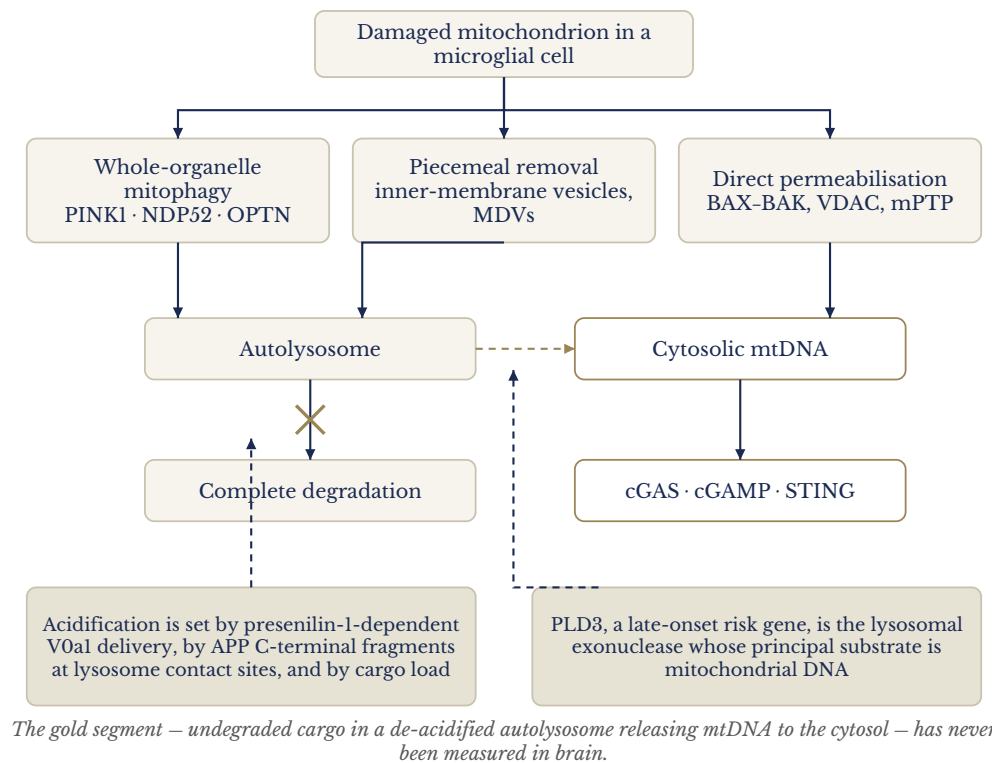


Figure 3. A damaged mitochondrion has three exits, and only the two on the left require the lysosome. The proposition that acidification failure backs up the left-hand routes and thereby raises cytosolic mtDNA is the load-bearing and least-tested claim in this review. PLD3, a late-onset risk gene, is a lysosomal exonuclease whose principal substrate is mitochondrial DNA. Sources: ^{120, 134, 142, 139}.

6.4 The joint: does lysosomal failure stall mitophagy in Alzheimer's disease?

This is the load-bearing claim of the review and it is the weakest. The proposition is stated in the major reviews of the field^{130,131} and it is mechanistically natural, since mitophagy terminates in the lysosome and a compartment that cannot acidify cannot complete a degradation. It has not been directly measured in Alzheimer's disease. The detailed characterisation of what accumulates in de-acidified autolysosomes reports amyloid- β , APP fragments and fibrillar material, and does not report mitochondrial cargo.¹²⁰ A search for undegraded mitochondria within de-acidified autolysosomes in Alzheimer's tissue or models returns nothing. The link is graded Tier 4.

Outside Alzheimer's disease the relationship is established and runs in both directions, which is itself a caution. Respiratory chain deficiency raises folliculin, deactivates AMP-activated protein kinase and thereby inhibits lysosomal hydrolysis, so mitochondrial failure causes lysosomal failure.¹³⁶ Mitochondrial reactive oxygen species cause lysosomal membrane permeabilisation.¹³⁷ Losing a lysosomal potassium channel degrades mitochondrial function.¹¹⁵ Tumour necrosis factor inhibits PINK1-mediated mitophagy and raises cytosolic mitochondrial DNA, so inflammation suppresses the clearance route.¹³⁸ Any linear reading — lysosome fails, therefore mitophagy stalls — is an arbitrary cut through a cycle with at least four documented arrows.

6.5 The one case where the joint has been demonstrated

There is a single study that does what the circuit requires, in a human-genetics-anchored system, and it deserves to be treated as the template for the missing experiment. *PLD3* polymorphisms are linked to late-onset Alzheimer's disease, and the protein is a lysosomal 5'–3' exonuclease whose principal physiological substrate was identified as mitochondrial DNA. In *PLD3*-defective cells, mitochondrial DNA builds up inside lysosomes and creates a proteolytic bottleneck, presenting ultrastructurally as multilamellar bodies often containing mitochondrial remnants, and correlating with **increased** PINK1-dependent mitophagy. Lysosomal leakage of mitochondrial DNA to the cytosol then activates cGAS–STING signalling, which upregulates autophagy and induces APP C-terminal fragment and cholesterol accumulation; STING inhibition largely normalises the APP fragments.¹³⁹

Three features of this result are decisive for the present argument. It demonstrates that a lysosomal defect can raise cytosolic mitochondrial DNA and activate cGAS–STING in a cell relevant to Alzheimer's disease. It shows the phenotype the circuit predicts — mitophagy initiation rising while completion fails — rather than a simple reduction in mitophagy. And it links that state to APP metabolism in a feedforward loop.

It also differs from the circuit in two respects that must not be elided. The lysosomal defect is a **nuclease** deficit, not an **acidification** deficit; and the cells are neuronal, not microglial. The study establishes that the joint can exist. It does not establish that it exists in the form this circuit needs. That is the experiment.

7. The Sensing Arm: Mitochondrial DNA and cGAS–STING

7.1 How mitochondrial DNA reaches a cytosolic sensor

Three routes are established. Following BAK and BAX activation, large outer-membrane macropores form and the inner membrane herniates into the cytosol carrying the genome.¹⁴⁰ Oxidatively stressed but non-apoptotic mitochondria release short fragments through oligomeric VDAC1 pores, and an inhibitor of that oligomerisation reduces release, interferon signalling and disease.¹⁴¹ Calcium uptake opens the permeability transition pore and triggers VDAC oligomerisation, with FEN1 cleaving oxidised mitochondrial DNA into fragments that exit and activate both the NLRP3 inflammasome and cGAS–STING — a result that unifies the two sensing arms around one species of ligand.¹⁴² Separately, aberrant nucleoid packaging from TFAM haploinsufficiency allows escape sufficient to engage cGAS–STING and produce an interferon-stimulated gene signature.¹⁴³

The pathway that receives the ligand is structurally defined. cGAS is the cytosolic DNA sensor that produces cGAMP,¹⁴⁴ and STING is the endoplasmic-reticulum adaptor that receives it.¹⁴⁵ Activation requires palmitoylation at the Golgi, making the pathway dependent on membrane trafficking.¹⁴⁶ Autophagy induction through STING trafficking is a primordial output of the pathway, independent of TBK1 and interferon.¹⁴⁷ Negative regulation is not optional: loss of the exonuclease TREX1 causes lethal cell-intrinsic autoimmunity,¹⁴⁸ and the lethality of DNase II deficiency is entirely STING-dependent.¹⁴⁹ Failure of nucleic acid clearance is sufficient to cause disease strictly through this pathway, which is the general form of the argument this review makes for Alzheimer's disease.

7.2 The pathway in Alzheimer's disease

The evidence here is the strongest in the circuit and includes human tissue. In a tauopathy model, pathogenic tau activates microglial cGAS and type I interferon in part through cytosolic leakage of mitochondrial DNA; ablating cGAS preserved synapses and memory without changing tau burden, restoring a neuronal MEF2C resilience programme, and pharmacological cGAS inhibition reproduced the rescue.¹⁵⁰ Deletion of *Sting1* in an amyloid model improved plaque control, reduced neuritic dystrophy and protected cognition, with reduced interferon gene expression in microglia and excitatory neurons.¹⁵¹ Inducible microglia-specific *Cgas* deletion in the same model restricted plaque accumulation and protected against cognitive impairment, establishing that the pathway's contribution is microglia-dominant.⁸ Amyloid and tau were shown to cause mitochondrial stress and cytoplasmic DNA release in microglia, with cGAS and STING high in plaque-associated microglia and neuronal STING upregulated in human Alzheimer's brain.¹⁵² Alzheimer's risk alleles amplify the effect: *APOE4* with *TREM2* R47H in female tauopathy mice increased microglial cGAS–STING and interferon signalling and produced senescence dependent on both cGAS and BAX, implicating mitochondrial permeabilisation upstream.¹⁵³ A herpesvirus challenge worsened tauopathy, and both *Sting* ablation and the inhibitor H-151 reduced neuroinflammation and tau pathology.¹⁵⁴

The most direct demonstration of the ligand step in human disease concerns neurons rather than microglia: glycated amyloid- β promotes neuronal mitochondrial DNA efflux through VDAC1, and cGAS–mitochondrial-DNA binding with pathway activation was observed in neuronal cytoplasm in aged model mice **and in human Alzheimer's brain samples**, with knockdown of RAGE, cGAS or STING and pharmacological VDAC1 inhibition all protective.¹⁵⁵ Metabolic context modulates the same axis: endothelial nicotinamide adenine dinucleotide depletion induces VDAC1 oligomerisation and mitochondrial DNA leakage driving senescence,¹⁵⁶ and nicotinamide riboside supplementation reduces neuroinflammation and senescence in an amyloid model through cGAS–STING.¹⁵⁷ Crosstalk with the inflammasome is direct: amyloid and mitochondrial DNA synergise through an mtDNA–STING–NLRP3–interleukin-1 β axis, and blocking any node interrupts it.¹⁵⁸

The ageing context is supplied by the demonstration that cGAS–STING drives ageing-related inflammation and neurodegeneration, with STING activation triggering reactive microglial states and cognitive decline,¹⁵⁹ built on the finding that cGAS senses cytosolic chromatin fragments in senescent cells and drives the senescence-associated secretory phenotype.¹⁶⁰ Most directly, microglia-specific deletion of *Tfam* in adult mice produces neuroinflammatory microglial states, cognitive ageing and neurodegeneration through cGAS–STING — microglia-intrinsic mitochondrial failure is sufficient.¹⁶¹

7.3 The output that matters for cognition

The interferon arm has been dissected by cell type. An interferon-stimulated gene signature is present across murine amyloid models, positive microglia localise selectively around nucleic-acid-containing plaques, and recombinant interferon- β drives complement C3-dependent synapse elimination in vivo.¹⁶² Conditional deletion separated the compartments: microglial *Ifnar1* deletion prevented post-synaptic engulfment, while neural *Ifnar1* deletion restored pre-synaptic terminals and reduced plaque load; long-term receptor blockade rescued memory and synaptic deficits.¹⁶³ The downstream effector machinery is the complement cascade that prunes synapses developmentally,¹⁶⁴ and that reappears perisynaptically in tauopathy and human Alzheimer's brain in proportion to phospho-tau.¹⁶⁵

The relevance of the terminal readout is not in question: synapse loss remains the strongest neuropathological correlate of cognitive impairment.¹⁶⁶

Neuronal STING is a distinct biology and not simply a spillover. In multiple sclerosis, glutamate excitotoxicity detaches neuronal STING from STIM1 and initiates non-canonical signalling that autophagically degrades GPX4 and causes ferroptosis.¹⁶⁷ Neuronal STING activation is present in human amyotrophic lateral sclerosis and frontotemporal dementia cortex and spinal motor neurons,

selectively in the most vulnerable populations.¹⁶⁸ Any intervention aimed at microglial STING in Alzheimer's disease will also act on this compartment.

7.4 Two results that a leakage model must accommodate

The first is a measurement problem that runs the wrong way. Cerebrospinal fluid cell-free mitochondrial DNA is **reduced** in asymptomatic at-risk individuals and in presymptomatic presenilin-1 mutation carriers,¹⁶⁹ and a systematic review finds the blood and cerebrospinal fluid mitochondrial DNA literature inconsistent across Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis.¹⁷⁰ If mitochondrial DNA were escaping cells in bulk, the naive expectation is the opposite.

The reconciliation available to this circuit is specific and testable rather than rhetorical. The circuit requires mitochondrial DNA in the **cytosol of microglia**, and that is a different quantity from cell-free mitochondrial DNA in **cerebrospinal fluid**. A peptide that condenses DNA and shields it from nuclease attack⁸⁴ would, if it operates in brain, reduce free extracellular DNA while increasing the intracellular sensed pool. The two observations are compatible only under that reading, which makes it a prediction rather than an excuse: a compartment-resolved measurement should show cytosolic microglial mitochondrial DNA rising while cell-free cerebrospinal fluid mitochondrial DNA falls. If both fall, the circuit is wrong.

The second is that human confirmation is thinner than the model literature suggests. Direct demonstration of cGAS–STING pathway activation in human post-mortem central nervous system across neurodegenerative diseases rests on a small series.¹⁷¹ No cGAMP measurement exists in human Alzheimer's cerebrospinal fluid or brain, and no STING or cGAS inhibitor has been tested clinically in any neurodegenerative disease.

7.5 The lysosome closes on the sensor as well as the ligand

A point that is easy to miss and that complicates every therapeutic proposal in this review: STING is terminated by autophagy–lysosomal degradation,¹⁷² so a degradative deficit prolongs the activated sensor as well as increasing its ligand. Both arms push in the same direction, which is convenient for the model and inconvenient for interpretation, because an intervention that improves degradation would act on both simultaneously and an experiment that measures only interferon output could not tell which arm moved.

STING is also not only an inflammatory protein. It is itself a proton channel whose transmembrane domain deacidifies post-Golgi vesicles and activates the v-ATPase–ATG16L1 axis for LC3 lipidation,¹⁷³ it activates TFEB and lysosomal biogenesis independently of TBK1,¹⁷⁴ and in lysosomal storage disorder brain it drives both a neuroinflammatory signature and TFEB-dependent lysosomal gene expression in microglia.¹⁷⁵ In a disease already characterised by lysosomal insufficiency, blanket STING inhibition would remove a lysosomal support function along with the inflammatory one. Lysosomal lipid accumulation in microglia is itself sufficient to activate STING and cause neurodegeneration,¹⁷⁶ so the two organelles are coupled through this pathway in both directions.

8. The Links, Taken One at a Time

Each of the fourteen links in Table 2 is argued here, so that the grading is auditable: for every link, what was observed, in what system, and what remains assumed.

8.1 E1 — LL-37 drives CLIC1 into the microglial membrane (Tier 2)

The claim is that an extracellular cationic peptide acts as an endogenous agonist for a metamorphic protein, promoting its translocation from cytosol and its integration into the plasma membrane, and thereby activating it.

The evidence is a single study of unusual scope. It reports the translocation and integration directly, attributes microglial hyperactivation, neuroinflammation and excitotoxicity to the activated protein, and demonstrates in mouse and monkey models that peptide exposure produces elevated amyloid- β , increased neurofibrillary tangles, neuronal death, brain atrophy, ventricular enlargement and impaired synaptic plasticity and cognition — with *Clc1* knockout and interaction blockade preventing each outcome.¹²

Biophysically the claim is unsurprising. CLIC1 insertion is promoted by oxidation³⁷ and depends on membrane cholesterol,³⁹ while protonation of specific histidines stabilises the intermediate state at acidic pH,⁴⁰ so a cationic amphipathic peptide that partitions into bilayers is a plausible trigger; LL-37 acts intracellularly through a defined partner⁷³ and permeabilises host membranes selectively by composition.¹⁷⁷

What is missing is replication. No independent laboratory has reproduced peptide-driven CLIC1 translocation in four years. The grade is Tier 2 on the strength of the in vivo genetic rescue, and would fall to Tier 3 if the effect proves species- or preparation-specific.

8.2 E2 — CLIC1 supports the microglial oxidative burst (Tier 3, mechanism contested)

Amyloid raises CLIC1 protein and a plasma-membrane conductance in neonatal rat microglia; block by four independent methods abolishes amyloid-induced reactive oxygen species; and the protein's own activation depends on oxidase-derived species, producing a feedforward loop.⁴⁴ Blocking the protein prevents neuronal apoptosis in co-culture and prevents amyloid-induced TNF- α release.⁴³ The effect is stimulus-selective, being absent for endotoxin with interferon- γ .⁴⁶

The mechanism is contested twice over. Surveillant microglia show no chloride conductance by patch clamp and the protein's function in them is chloride-independent, running through ezrin-radixin-moesin proteins.⁴ In macrophages the null phenotype is failure of NADPH oxidase to redistribute to the plasma membrane rather than loss of an anion current.⁴⁹ The system is also the weakest available: cultured neonatal rodent microglia lose the transcriptional identity that defines the cell.¹⁶

The node survives the correction; the charge-balance mechanism may not. Tier 3.

8.3 E3 — CLIC1 licenses inflammasome output (Tier 2)

In microglia, CLIC1 blockade after ATP-evoked activation strongly suppresses NLRP3-dependent interleukin-1 β release.⁴ In macrophages, CLIC-dependent chloride efflux is an essential proximal upstream event for NLRP3 activation with a fully ordered sequence,⁵⁶ the proteins act at the priming step as well,⁵⁷ and extracellular acidosis drives translocation and amplifies caspase-1 processing.⁵⁸ The relevance of the output to Alzheimer's disease is established independently: fibrillar amyloid activates NLRP3 through lysosomal damage and cathepsin release, and the inflammasome is required for microglial recruitment to amyloid in brain.¹⁷⁸

This is the best-supported CLIC1 link and the one with the clearest therapeutic readout. Tier 2.

8.4 E4 — CLIC1 supports phagosomal acidification and proteolysis (Tier 3)

CLIC1 translocates onto the phagosomal membrane during phagocytosis; null macrophages show defective phagosomal acidification with intact phagosome–lysosome fusion, impaired proteolytic capacity and reduced reactive oxygen species, and the animals are protected from inflammatory arthritis.⁵¹ The mechanism reproduces in dendritic cells with consequences for antigen processing and for autoimmune encephalomyelitis.⁵²

The system is myeloid, which is the right lineage, but neither cell is a microglial cell and neither compartment is a lysosome. Tier 3.

8.5 E5 — CLIC1 supports lysosomal acidification (Tier 0)

No study has tested CLIC1 as a counterion conductance at the lysosome in any system. The thermodynamic requirement for a counterion is established,⁵³ the canonical candidates are the CLC transporters,⁵⁵ and CLIC1 is not among them. The phagosomal result is suggestive because a phagosome matures into a degradative compartment, but suggestion is not measurement — and the phagosomal study explicitly found fusion unimpaired, meaning the defect was in the acidification machinery of a compartment that had already received lysosomal content.⁵¹

This is one of two places where the circuit is held by an assumption. It is graded Tier 0 and it is an experiment, not a conclusion.

8.6 E6 — Microglial oxidative and inflammatory load damages microglial mitochondria (Tier 3)

Mitochondrial reactive oxygen species cause lysosomal membrane permeabilisation and NLRP3-dependent damage in macrophages,¹³⁷ and tumour necrosis factor inhibits PINK1-mediated mitophagy while raising cytosolic mitochondrial DNA.¹³⁸ In microglia, amyloid triggers an mTOR- and HIF-1 α -dependent switch from oxidative phosphorylation to glycolysis followed by a chronic tolerant state with broad energy-metabolism defects and diminished phagocytosis,¹⁷⁹ and mitochondrial state gates phagocytic competence directly.⁷

The microglial metabolic literature is Alzheimer's-specific; the mitochondrial-damage-to-lysosomal-damage literature is not. Tier 3.

8.7 E7 — Lysosomal acidification failure stalls mitophagy in Alzheimer's disease (Tier 4)

This is the joint on which the circuit turns, and no one has measured it. The proposition is asserted in the principal reviews,^{130,131} and the acidification deficit itself is well demonstrated in neurons.^{118,120} But the detailed characterisation of de-acidified autolysosome content does not report mitochondrial cargo,¹²⁰ there is no flux measurement in human brain, and static markers cannot separate reduced initiation from failed completion. The reverse causality is at least as well documented as the forward one.¹³⁶

The nearest demonstration is the *PLD3* work, in which a lysosomal defect produces exactly the predicted signature — a proteolytic bottleneck with multilamellar bodies containing mitochondrial remnants and **increased** PINK1-dependent mitophagy — but the defect is nucleolytic rather than acidic, and the cells are neuronal.¹³⁹ Tier 4.

8.8 E8 — Stalled mitophagy raises cytosolic mitochondrial DNA (Tier 3; anchor retracted)

The most-cited demonstration of this relationship — that parkin and PINK1 mitigate STING-induced inflammation, with inflammation after exhaustive exercise or mitochondrial DNA mutation rescued by STING deletion — has been **retracted**.¹⁸⁰ The record is flagged as a retracted publication in the bibliographic database. It is cited here only to record that it has been withdrawn, because a substantial downstream literature rests on it and a mechanistic review that quietly substituted a different citation would misrepresent the strength of the field.

What remains is real but peripheral, and the strongest study runs the argument in the gain direction rather than the loss direction: pharmacological mitophagy induction suppresses cytosolic mitochondrial-DNA-dependent cGAS/STING activation in ageing tissue, which implies rather than demonstrates that insufficient mitophagy permits it.¹³³ Ageing promotes cytosolic mitochondrial DNA leakage in macrophages with enhanced STING activation, abrogated by mitochondrial DNA depletion.¹⁸¹ Impaired mitophagy execution produces a type I interferonopathy in a genetic model.¹⁸² Inflammatory signalling suppresses mitophagy and raises cytosolic mitochondrial DNA.¹³⁸ In brain, the closest primary result is a manganese parkinsonism model in which suppression of microglial PINK1–parkin mitophagy raises cytosolic mitochondrial DNA and STING signalling, and in which a mitophagy inducer reverses the microglial activation.¹⁸³ A recent review frames microglial mitophagy as an immunometabolic checkpoint restraining both cGAS–STING and NLRP3.¹⁸⁴

Tier 3, and weaker than the field's citation pattern implies.

8.9 E9 — Cytosolic mitochondrial DNA activates cGAS–STING in Alzheimer's disease (Tier 1)

Tau activates microglial cGAS and interferon in part through cytosolic mitochondrial DNA leakage, with cGAS ablation preserving synapses and memory.¹⁵⁰ Amyloid and tau cause mitochondrial stress and cytoplasmic DNA release in microglia, with pathway components high in plaque-associated microglia and neuronal STING upregulated in model brain.¹⁵² And cGAS–mitochondrial-DNA binding with pathway activation has been observed in human Alzheimer's brain samples alongside aged model mice, with VDAC1 identified as the efflux route.¹⁵⁵

This is the only Tier 1 link in the circuit, and the qualification made in Chapter 7 applies: the human-tissue observation is neuronal, while the circuit argued here is microglial. The microglial version of this link is Tier 2.

8.10 E10 — cGAS–STING output removes synapses (Tier 2)

Interferon-stimulated-gene-positive microglia localise around nucleic-acid-containing plaques and interferon- β drives complement-dependent synapse elimination,¹⁶² with cell-type-resolved conditional deletion separating pre- from post-synaptic effects and long-term receptor blockade rescuing memory.¹⁶³ Genetic removal of STING or of microglial cGAS protects cognition in amyloid models.¹⁵¹⁸ The effector arm is the complement machinery that prunes synapses and reappears in tauopathy and human Alzheimer's tissue.¹⁶⁴¹⁶⁵

Tier 2, with strong convergence across laboratories and models.

8.11 E11 — Interferon and infection raise CAMP transcription (Tier 3) — closure 1

Interferon- γ raises LL-37 in human astrocytes and endotoxin with interferon- γ raises it in human microglia.⁵ Toll-like receptor activation drives the vitamin-D-dependent induction in human macrophages.⁷⁵ Amyloid- β itself induces the murine orthologue in microglia.⁹⁴ The peptide is detectable in human cerebrospinal fluid during central nervous system infection.⁹³

This closure has a structural caveat that cannot be engineered away: the vitamin-D arm of the induction is primate-specific in origin,⁷⁶ so a mouse cannot test it. Tier 3.

8.12 E12 — Mitochondrial reactive oxygen species drive CLIC1 insertion (Tier 3) — closure 2

The inflammasome ordering places mitochondrial damage and mitochondrial reactive oxygen species upstream of CLIC translocation to the plasma membrane.⁵⁶ Oxidation is the canonical trigger for the metamorphic transition,³⁶³⁷ CLIC1 is among the cysteine-redox-modified proteins in amyloid-challenged microglia,⁴⁷ and hydrogen peroxide promotes CLIC1 translocation and membrane insertion in endothelial cells with the classical blocker preventing it.¹⁸⁵

This is the closure that converts the arrangement from a cascade into a loop, because it makes the downstream mitochondrial lesion a cause of the upstream channel event. It has not been demonstrated in microglia. Tier 3.

8.13 E13 — LL-37 carries nucleic acid and messenger into the cytosol (Tier 3)

LL-37 transports self-DNA into human primary monocytes, producing type I interferon in a Toll-like-receptor-independent manner that requires STING and TBK1.⁸² The peptide also binds and transports extracellular cGAMP into cells to activate STING.⁸³ A complete version of the mechanism exists in mast cells, where the peptide causes reactive-oxygen-driven mitochondrial damage and permeability transition opening with cytosolic mitochondrial DNA leakage and cGAS/STING activation,⁸⁹ and an independent line reports peptide-induced STING-mediated inflammation in skin macrophages.⁹⁰

Every one of these systems is peripheral. No study has examined the human peptide's relationship to cGAS or STING in a central nervous system cell, and the single central nervous system result uses the murine orthologue and runs the other way: it **suppresses** microglial cGAS–STING signalling after facial nerve injury.¹⁸⁶ Tier 3, with an unexplained sign conflict in the only brain datapoint that exists.

8.14 E14 — LL-37 stabilises mitochondrial DNA as a persistent ligand (Tier 3)

LL-37–mitochondrial DNA complexes are elevated in human atherosclerotic plasma and plaque, resist DNase II degradation and escape autophagic recognition; the murine equivalent worsens lesions and an antibody against the complex rescues.⁸⁴ The resistance mechanism was reproduced in septic lung.⁸⁵ The peptide plus mitochondrial DNA induces spontaneous interferon production from human plasmacytoid dendritic cells,⁸⁶ and oxidative modification increases the stimulatory capacity of the DNA.⁸⁷

This link matters disproportionately because it is the one that could reconcile the cerebrospinal fluid measurement problem: a ligand that is shielded from nuclease and hidden from autophagy would be simultaneously more persistent inside cells and less abundant as free extracellular DNA. The complex has never been looked for in brain or cerebrospinal fluid. Tier 3.

9. The Circuit Assembled

9.1 Statement of the model

The proposition, stated in full and with its status attached, is this.

In a primed microglial cell, cathelicidin generated locally under interferon, endotoxin or amyloid drive acts on CLIC1, promoting its conversion from the soluble cytosolic form to the membrane-inserted form. The inserted protein organises the membrane machinery that sustains an oxidative burst and licenses inflammasome assembly. The oxidative and degradative load produced falls simultaneously on two compartments that are already compromised in Alzheimer's disease: the mitochondrion and the lysosome. Because mitochondrial quality control terminates in the lysosome, a compartment that cannot complete degradation converts mitophagy from a clearance process into a holding pattern, in which initiation continues and completion does not. Mitochondrial DNA that is neither destroyed nor contained reaches the cytosol, where cGAS produces cGAMP and STING converts a metabolic failure into a sustained type I interferon signal. That signal removes synapses through the complement machinery, and it also raises transcription of the gene encoding the peptide that began the sequence. Separately, the mitochondrial reactive oxygen species generated in the middle of the sequence are themselves a principal trigger for the channel insertion at its start. The peptide additionally acts at the sensing step directly, shielding mitochondrial DNA from nuclease attack and carrying nucleic acid and cyclic dinucleotide across the membrane to the cytosolic sensor.

Of the fourteen links this requires, one is supported in human Alzheimer's tissue, three in disease models, eight by cross-system inference, one is an untested prediction, and one has never been examined in any system.

9.2 What the two closures buy, and what they cost

A linear cascade requires its initiating stimulus to persist; a circuit does not. If interferon output raises peptide production, and if the mitochondrial damage in the middle of the sequence promotes the channel insertion at its start, then a transient event — an infection, a period of systemic inflammation, a vascular insult — can install a state that outlives it. This supplies what a model of a slowly progressive disease most needs: an explanation of why the process continues after its cause has gone.

The cost is that self-sustaining models are unfalsifiable if stated loosely. A circuit that maintains itself predicts specifically that removing the initiating stimulus after establishment will not stop the process, while interrupting any interior node will. That asymmetry is testable, and Chapter 13 operationalises it as a pulse-and-withdrawal design. A model that cannot name the node which must remain engaged for injury to continue is not making a claim.

9.3 Where the circuit is genuinely novel, and where it is not

Two of its segments are not new. That mitochondrial DNA activates cGAS–STING in Alzheimer's disease, and that the resulting interferon removes synapses, is an established and actively worked area with human tissue support. Nothing in this review improves on it.

What is proposed here is an upstream arm and a coupling. The upstream arm is that a host-defence peptide and a metamorphic membrane protein constitute a redox-gated entry point into microglial activation — a pairing established in one study that the Alzheimer's literature has largely not absorbed,¹² built on a CLIC1 microglial literature that is twenty years old and was substantially revised in 2025.⁴ The coupling is that the degradative compartment is the junction between the two arms: CLIC1 is known to support acidification of a degradative compartment in myeloid cells,⁵¹ and acidification failure is the established lesion in Alzheimer's disease.¹¹⁸¹²⁰

That coupling is also where the evidence is thinnest. The two claims it needs — that CLIC1 acts at the lysosome (E5) and that acidification failure stalls mitophagy in this disease (E7) — are graded Tier 0 and Tier 4 respectively. The novelty and the weakness are located in the same place, which is the honest description of the situation.

9.4 What the model predicts that existing models do not

Three consequences distinguish this circuit from the standard mitochondrial-DNA–interferon account.

First, it predicts that the microglial interferon response in Alzheimer's disease has an **extracellular, peptide-dependent entry point** that can be blocked without touching the sensor. If so, an intervention at CLIC1 should reduce interferon output while leaving the cell's capacity to respond to genuine cytosolic DNA intact — a selectivity that STING inhibition cannot offer.

Second, it predicts that the degradative deficit and the inflammatory output are **not two parallel problems but one problem read at two points**, which implies that an intervention improving lysosomal completion should reduce interferon output, and that an intervention reducing interferon output should improve lysosomal load. Neither implication has been tested in the same experiment.

Third, it predicts a **specific compartmental dissociation** in the measurement of mitochondrial DNA: rising in the microglial cytosol, falling as free nucleic acid in cerebrospinal fluid, because the same peptide that delivers it inward also protects it from the nucleases that would otherwise release fragments into the extracellular pool. This is the least intuitive prediction in the review and the one most capable of killing it.

9.5 Therapeutic geometry, stated as consequence rather than proposal

The review was not written to nominate a drug, and the following is offered only as what the model entails.

The node with the most favourable properties is CLIC1. Its constitutive knockout is viable, fertile and grossly normal with a mild platelet defect as the only baseline abnormality,⁶⁸⁶⁹ while the same animals are protected across four independent inflammatory challenges and in peptide-driven Alzheimer's-like pathology.¹² It is already inhibited by a licensed medicine through a defined residue,⁶⁴⁶⁶ and a second chemotype blocks the conformational transition rather than the pore.⁶⁷ The disease-associated species is the membrane-inserted form rather than total protein, and it is detectable in patient cells,⁵⁹ which supplies a pharmacodynamic marker.

The node with the least favourable properties is STING, for reasons that are structural rather than practical. STING supports lysosomal biogenesis independently of its inflammatory signalling,¹⁷⁴ performs lysosomal quality control in microglia in lysosomal disease brain,¹⁷⁵ and acts through a proton channel that engages the autophagy machinery.¹⁷³ Inhibiting it in a disease of lysosomal insufficiency removes a support function together with the pathological one. Direct evidence that this concern is not theoretical comes from the finding that a STING **agonist** improved cognition and reduced plaque burden in an amyloid model, discussed in the next chapter.

The lysosomal node is attractive because it sits at the junction, and is complicated for the same reason. Restoring acidification is pharmacologically achievable in vivo,¹²³ but acidification is a defended set-point in which excess is as pathological as deficiency,¹¹³¹¹⁴ and phagolysosomal acidification is **required** for the membrane permeabilisation that drives particle-induced inflammasome activation.¹¹ In a plaque-laden brain, re-acidifying microglial lysosomes might amplify interleukin-1 β rather than resolve it. That is a genuine contradiction between the neuronal and microglial arms of the same hypothesis and it is not resolved here.

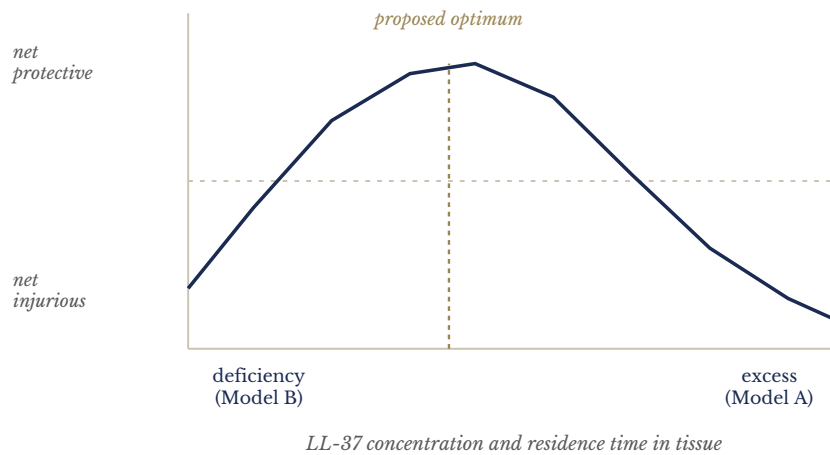
10. Evidence Against the Model

A review that reports only the evidence assembling its circuit is an advocacy document. This chapter collects the findings that cut against it, in descending order of how much damage they do.

10.1 The peptide has the wrong sign in half the literature

This is the most serious problem and it is not a matter of study quality. One programme holds that LL-37 in excess drives Alzheimer's-like pathology through CLIC1.¹² Another holds that Alzheimer's disease follows in part from the **destruction** of LL-37 by bacterial proteases, removing a peptide that binds amyloid- β and restrains its assembly and that contributes to antiviral defence.¹¹⁰ In vitro the peptide inhibits amyloid fibril assembly¹⁰² and forms off-pathway hetero-oligomers with amyloid- β ,¹⁰³ which supports the second model and sits awkwardly with the first, where peptide exposure *raised* amyloid in vivo.

In brain the directional evidence is split down the middle. Cathelicidin deficiency worsens bacterial meningitis outcome,⁹⁵ intraventricular peptide reduces mortality and glial activation,⁹⁶ and exogenous peptide inhibits endotoxin-driven glial cytokine production.⁹⁷ Against this, cathelicidin drives astrocyte–microglia neuroinflammation in autoimmune encephalomyelitis with corroborating human tissue,⁹⁸ and drives severe disease through Th17 differentiation in the same model.⁹⁹ The pattern across all tissues is that the peptide is protective in acute infection and injurious in chronic sterile inflammation, but that is a description rather than a mechanism.



At low occupancy the peptide binds amyloid- β , restrains fibril assembly and supports microbial containment. At high occupancy it drives CLIC1 insertion, stabilises mitochondrial DNA against nuclease attack and carries it to the cytosolic sensor. The framework predicts an interior optimum that can be measured.

Figure 5. Author-proposed analytical framework, not measured data. A single non-monotonic dose-effect relation reconciles a peptide that binds and restrains amyloid at low occupancy with one that drives channel activation and sustained inflammation at high occupancy. The framework is falsifiable: it requires a measurable interior optimum. Sources: ^{102, 12, 109}.

A biphasic reading resolves the contradiction formally. At low occupancy the peptide binds amyloid- β and restrains fibril assembly while supporting microbial containment; at high occupancy it drives channel insertion, stabilises mitochondrial DNA against nuclease attack, and carries nucleic acid to the cytosolic sensor. Both models would then be describing the same curve from opposite sides. The reading is proposed as an analytical framework, not as a finding, and it is falsifiable: it requires a measurable interior optimum. A dose-response experiment spanning at least three log units of peptide concentration in a single microglial preparation, with amyloid handling and interferon output measured together, would find that optimum or fail to. A monotonic result in either direction falsifies the framework.

Two further complications should be carried with it. The peptide is not one molecule — citrullination abolishes its DNA-delivery function,⁷⁷ and neutrophil-derived and macrophage-derived forms behave differently.⁷⁸ And the only measurement of the peptide against human cognitive outcomes is partly null: serum LL-37 showed no association with continuous cognitive slope, with the supportive findings confined to secondary endpoints.¹⁰⁹

10.2 Cell-free mitochondrial DNA is reduced in Alzheimer's disease, not raised

Cerebrospinal fluid cell-free mitochondrial DNA is reduced in asymptomatic at-risk individuals and in presymptomatic presenilin-1 mutation carriers,¹⁶⁹ and the fluid mitochondrial DNA literature across neurodegenerative diseases is systematically inconsistent.¹⁷⁰ A model whose central event is mitochondrial DNA escaping into the cytosol must explain why the accessible biomarker moves the other way.

The explanation offered in Chapter 7 — that cytosolic and extracellular pools are different quantities, and that a DNA-condensing, nuclease-shielding peptide would drive them in opposite directions — is coherent. It is also exactly the kind of explanation that rescues a model from its own disconfirmation, and deserves suspicion until tested. The currently measurable quantity does not support the model, and the model survives only by asserting that the measurable quantity is the wrong one. That assertion is prediction P6.

10.3 A STING agonist improved an amyloid model

The therapeutic logic of the sensing arm assumes that pathway activation is injurious. One study reports that the STING agonist cGAMP *ameliorated* cognitive deficits, reduced amyloid plaque load and reduced neuronal apoptosis in an amyloid model by inducing TREM2 and shifting microglial polarisation.¹⁸⁷ This is a head-on contradiction of the knockout and inhibitor literature in the same model. Separately, STING-dependent type I interferon has been shown to *reduce* microglial reactivity and neuroinflammation.¹⁸⁸

These results are compatible with the pathway being dose- and duration-dependent rather than directionally fixed — transient activation supporting antiviral defence, autophagy and quality control; sustained activation driving injury. If that is right, then the model's claim must be narrowed from "pathway activation is pathological" to "failure of pathway termination is pathological", which changes the therapeutic target from the sensor to its removal machinery.

10.4 The most-cited mitophagy-to-STING result has been retracted

The paper establishing that parkin and PINK1 restrain STING-induced inflammation, and that dopaminergic loss in a mitochondrial-mutation model is rescued by STING deletion, is flagged as a retracted publication.¹⁸⁰ Link E8 accordingly rests on a set of peripheral studies in ageing macrophages, retina, arthritis and a genetic interferonopathy, none in brain and none in Alzheimer's disease. The field's citation pattern does not reflect this, and any confidence in E8 calibrated to how often the relationship is asserted is miscalibrated.

10.5 The CLIC1 mechanism was substantially revised in 2025

Two decades of microglial CLIC1 work rests on a chloride conductance that patch-clamp recording does not detect in surveillant microglia, in a study that also finds the protein's function chloride-independent and mediated through cytoskeletal adaptors.⁴ In macrophages, the null phenotype is a trafficking failure rather than loss of an anion current.⁴⁹ The node survives; the mechanism attributed to it for twenty years may not. Any experiment designed around a chloride readout is likely to be measuring the wrong thing.

10.6 Mitophagy may not decline, and bulk autophagy is not a proxy for it

In reporter mice across multiple organs, mitophagy in old animals is increased or unchanged rather than decreased.¹³³ Basal mitophagy in neural tissue does not require PINK1.¹³² Sub-organellar removal routes operate constitutively and are invisible to standard reporters.^{134,135} And stimulating autophagy in an amyloid context can be harmful: autophagic vacuoles are themselves an amyloid-generating compartment,¹⁸⁹ and inducing autophagy in mutant APP neurons accumulates BACE1 in distal axons and augments β -cleavage.¹⁹⁰

Together these mean that "mitophagy is impaired in Alzheimer's disease" may be both true and misleading — true as a statement about completion, misleading as a statement about flux — and that the intervention implied by the model is lysosomal completion rather than autophagy initiation.

10.7 The microglial literature contradicts itself on the most basic manipulation

Microglial depletion prevents neuronal loss without changing amyloid;³⁰ sustained early depletion prevents plaque formation entirely;³¹ four-week depletion changes neither plaque formation nor maintenance nor neuritic dystrophy.³² Microglial envelopment of plaques is protective, compacting them and insulating neurites, with barrier coverage declining with age.¹⁹¹ TREM2 loss worsens amyloid pathology in one body of work¹⁹² and ameliorates both amyloid and tau pathology in another,¹⁹³ and protects against neurodegeneration in tauopathy.¹⁹⁴ An interferon-linked microglial state can be protective, enhancing plaque compaction and improving cognition.¹⁹⁵ Blocking type I interferon signalling *increases* microglial amyloid uptake.¹⁹⁶

None of this refutes the present circuit directly. All of it means that the sign of any microglial intervention is stage-dependent and that a model predicting uniform benefit from microglial quieting is wrong on the existing evidence.

10.8 Human neuropathology supports failure rather than overactivation

Microglia adjacent to tau pathology in human brain are dystrophic rather than activated, and microglial dystrophy correlates in situ with neurofibrillary degeneration.²⁵²⁶ The protective human microglial subset is reduced in frequency in disease rather than a disease subset being gained.²⁴ Human microglial signatures in Alzheimer's disease do not reproduce the mouse disease-associated programme.²²

The circuit proposed here is compatible with the exhaustion framing — a cell running an oxidative burst it cannot sustain, with a degradative compartment it cannot complete, is a cell on the way to dystrophy — but that compatibility was not designed in, and the circuit as drawn describes a hyperactive cell. Reconciling the two would require showing that the circuit's early phase is hyperactivity and its late phase is failure, which is a claim about time course that no study in this literature has made.

10.9 A pathway can be activated without causing the damage attributed to it

In a model of Aicardi–Goutières syndrome, cerebellar neuropathology was rescued by deleting p53 but **not** by deleting cGAS, indicating that the neuropathology tracked DNA-damage signalling rather than cGAS-driven neuroinflammation.¹⁹⁷ The general lesson is directly applicable: demonstrating that cGAS–STING is activated in Alzheimer's brain, which has been done, does not demonstrate that its activation causes the injury. The genetic rescues in amyloid and tau models are what carry that claim, and they carry it in mice.

11. Relationships That Do Not Exist in the Literature

The instruction under which this review was written was not to force connections. Ten relationships among these seven elements were searched for directly. Nine return no primary literature at all and the tenth returns a single study in the wrong species running in the wrong direction. They are reported here as findings, because an absence that is named is usable and an absence that is passed over is not.

Each entry states the search and what its emptiness costs the model. The claim is about the indexed literature as of September 2026, not about biology: a Tier 0 link may be true, untested, or untestable, and the three are not distinguished by a null search.

Table 3 — Ten searched relationships with no supporting primary literature, and the consequence of each absence. The ninth row is the one partial exception.

Relationship searched	Result	What the absence costs
CLIC1 and mitophagy	No paper in any system	The channel arm cannot be connected to the quality-control arm directly; the connection must run through acidification, which is itself untested
CLIC1 and cGAS or STING	No paper; search returns only false positives	The two arms of the circuit have no direct molecular link; they meet only at the degradative compartment
CLIC1 and type I interferon	No mechanistic paper; five incidental co-occurrences	The channel has never been placed in the interferon pathway that the circuit's output requires
CLIC1 and lysosomal acidification	No paper; evidence is phagosomal only	Link E5, the junction between the arms, is unmeasured
CLIC1 in human Alzheimer's brain tissue	No immunohistochemistry or tissue proteomics	The transducing node has never been shown to be present, let alone inserted, in the disease
CLIC1 with TREM2, APOE or tau	No mechanistic paper	The channel is unconnected to the disease's principal genetic and pathological axes
LL-37 and mitophagy	No paper in any system	The peptide's documented interference with autophagic recognition of mitochondrial DNA has never been extended to the mitophagy route itself
LL-37 or its precursor in human Alzheimer's brain	Never measured	The initiating node has never been shown to be present in the disease tissue
LL-37 and cGAS or STING in a CNS cell	One paper, murine orthologue, opposite sign	Link E13 rests otherwise on monocytes, mast cells and skin macrophages
LL-37-mitochondrial DNA complexes in brain or CSF	No paper	Link E14, which alone reconciles the cerebrospinal fluid measurement problem, is unexamined in the relevant compartment

Three further absences are worth recording separately because they bear on interpretation rather than on a specific link.

No variant of *CAMP*, the gene encoding LL-37, has been associated with dementia risk. For a molecule proposed as an upstream driver in a disease whose risk architecture is substantially myeloid and well mapped, the absence of a genetic signal is a real argument against a primary causal role, though not against a modifying one. The same is true of *CGAS* and *STING1*, neither of which appears among Alzheimer's risk loci despite the pathway's prominence in the mechanistic literature.

No clinical trial of any cGAS or STING inhibitor has been conducted in Alzheimer's disease or in any neurodegenerative disease. No completed trial of a selective mitophagy activator such as urolithin A exists in Alzheimer's disease with cognitive endpoints, although the class has human safety and pharmacodynamic data in healthy and older adults,¹⁹⁸ including evidence that a mitophagy inducer remodels human immune cells.¹⁹⁹ The adjacent clinical evidence is mixed rather than absent: a phase II trial of combined metabolic activators reported improved cognitive function,²⁰⁰ while a phase II trial of the nicotinamide adenine dinucleotide precursor nicotinamide riboside in amnesic mild cognitive impairment raised blood levels twofold and produced no improvement in its primary cognitive outcome.²⁰¹ Any claim that mitophagy activation has established clinical benefit in Alzheimer's disease is unsupported.

No study has demonstrated the macropore or herniation route for mitochondrial DNA release in brain tissue of any Alzheimer's model. The routes implicated in the disease are VDAC1-dependent;¹⁵⁵¹⁵⁶ the permeability transition route is established in an amyotrophic lateral sclerosis model and in macrophages²⁰²¹⁴² but has not been tested in Alzheimer's disease.

The distribution of these absences is itself informative. They cluster almost entirely on the CLIC1 and LL-37 nodes — that is, on the arm this review proposes — while the mitochondrial DNA-sensor arm is comparatively well populated. The upstream half of the circuit is not contested; it is unexamined. That is a better position to be in than contradicted, and a worse one than supported.

12. Falsifiable Predictions

Each prediction below states what the circuit requires, what observation would confirm it, and what observation would falsify it. Predictions are ordered by how much of the model each one decides. Confirmation of any single prediction does not establish the circuit; falsification of P1, P2, P4 or P7 removes it.

P1 — The initiating molecule is present in the diseased tissue. *Requires:* LL-37 or hCAP-18 detectable in human Alzheimer's brain, at levels exceeding age-matched control tissue, and localised to or adjacent to microglia. *Falsified by:* absence of detectable peptide, or no difference from control. This has never been measured. The only demonstration of the peptide in human central nervous system fluid is in acute bacterial meningitis.⁹³ Its expression in human microglia and astrocytes under inflammatory stimulation is established in culture.⁵ The measurement is straightforward and it is the cheapest way to end this enquiry.

P2 — The transducing protein is in the membrane, not merely present. *Requires:* the membrane-inserted CLIC1 species increased in microglia in human Alzheimer's brain relative to control, with total CLIC1 either unchanged or less changed than the inserted fraction. *Falsified by:* no shift in the inserted fraction. The distinction between total and inserted protein is the one that matters,⁶⁰ the inserted species is detectable in patient cells by electrophysiology and conformation-selective antibody,⁵⁹ and CLIC1 is microglia-enriched in human brain tissue.⁴ No one has made this measurement in Alzheimer's tissue.

P3 — The channel's microglial function is not a chloride current. *Requires:* CLIC1-dependent effects in microglia persisting under anion substitution, and tracking membrane insertion and cytoskeletal association rather than conductance. *Falsified by:* recovery of a CLIC1-attributable

chloride conductance in activated adult microglia that accounts for the functional effects. This prediction follows the 2025 revision rather than the older literature,⁴⁴⁹ and it is included because it determines what every subsequent experiment should measure.

P4 — Mitophagy in Alzheimer's microglia stalls at completion, not at initiation. *Requires:* in a model with both amyloid and tau, microglial mitophagy initiation markers unchanged or increased while flux to degradation is reduced, with undegraded mitochondrial material demonstrable inside de-acidified autolysosomes. *Falsified by:* reduced initiation with preserved flux, or normal flux at the stage when cytosolic mitochondrial DNA rises. This is the load-bearing prediction. The signature it describes — initiation up, completion blocked — is exactly what a lysosomal nuclease deficit produced in a different system.¹³⁹ The characterisation of de-acidified autolysosome content in Alzheimer's models does not report mitochondrial cargo,¹²⁰ so the prediction is currently unsupported by the best available dataset.

P5 — The junction is causal in the direction claimed. *Requires:* restoring lysosomal acidification in microglia reduces cytosolic mitochondrial DNA and interferon-stimulated gene induction. *Falsified by:* acidification rescue that leaves cytosolic mitochondrial DNA unchanged, or that raises interleukin-1 β without lowering interferon output. Acidification is pharmacologically correctable in vivo,¹²³ but the falsifying outcome is a live possibility rather than a formality: phagolysosomal acidification is required for the membrane permeabilisation that drives particle-induced inflammasome activation,¹¹ and amyloid activates the inflammasome by exactly that route.¹⁷⁸

P6 — The two mitochondrial DNA pools move in opposite directions. *Requires:* microglial cytosolic mitochondrial DNA rising with disease stage while cell-free mitochondrial DNA in cerebrospinal fluid falls, with LL-37-mitochondrial DNA complexes detectable in brain or cerebrospinal fluid. *Falsified by:* both pools falling, or complexes undetectable while cytosolic mitochondrial DNA rises. This is the prediction that addresses the model's most direct disconfirmation.¹⁶⁹¹⁷⁰ The complex has been demonstrated in human plasma and plaque in a different disease and is nuclease-resistant and autophagy-evading,⁸⁴ but has never been sought in brain.

P7 — The upstream arm is necessary for the downstream arm. *Requires:* microglia-specific deletion of *Clic1* in a model carrying both amyloid and tau reduces cytosolic mitochondrial DNA, interferon-stimulated gene induction and synapse loss. *Falsified by:* no change in cytosolic mitochondrial DNA or interferon output despite effective deletion. The systemic knockout is protected in peptide-driven pathology,¹² but that experiment does not establish cell autonomy, does not use a standard amyloid or tau model, and does not measure the sensing arm. Falsification here would mean the two arms are parallel rather than serial, which would leave the sensing arm intact as established biology and remove the circuit.

P8 — The circuit maintains itself after its initiator is withdrawn. *Requires:* after a defined pulse of peptide exposure and its withdrawal, interferon output and cytosolic mitochondrial DNA remain elevated, and interruption of an interior node reverses them. *Falsified by:* return to baseline on withdrawal. This is what distinguishes a circuit from a cascade and it has not been attempted for any part of this arrangement.

P9 — Closure 2 operates in microglia. *Requires:* scavenging mitochondrial reactive oxygen species reduces CLIC1 membrane insertion in activated microglia. *Falsified by:* insertion unchanged by mitochondrially targeted antioxidant. The ordering is established in macrophages for the inflammasome,⁵⁶ oxidation is the canonical insertion trigger,³⁶³⁷ and hydrogen peroxide drives insertion in endothelial cells,¹⁸⁵ but the specific mitochondrial source has not been tested in microglia.

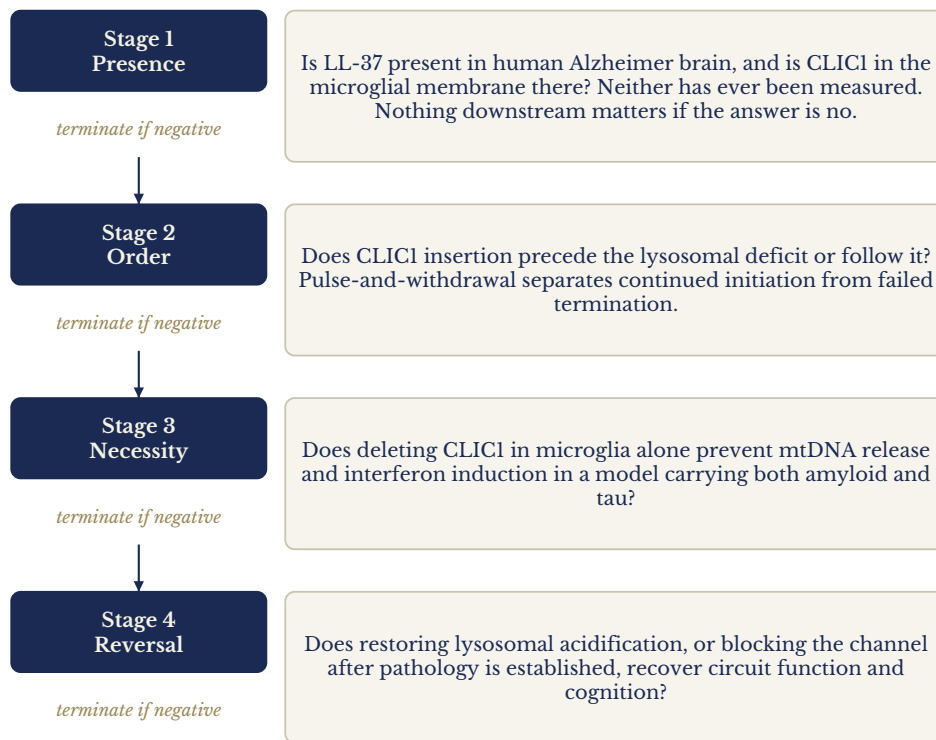
P10 — The peptide's effect is non-monotonic. *Requires:* across at least three log units of concentration in one microglial preparation, an interior optimum in which amyloid handling is preserved and interferon output is low. *Falsified by:* a monotonic dose-response in either direction.

This is the discriminating experiment for the contradiction in Chapter 10.1, and it is the only prediction here that would adjudicate between two existing programmes rather than test a new claim.

P11 — The sign conflict in brain is compartmental. *Requires:* the peptide to act on cGAS–STING in the direction reported peripherally when delivered to the microglial cytosol, and in the suppressive direction reported in brain when acting extracellularly.⁸²¹⁸⁶ *Falsified by:* consistent suppression regardless of compartment, which would remove link E13.

13. A Staged Experimental Programme

The programme is ordered so that the cheapest and most decisive measurement comes first, and so that a negative result at any stage terminates the remainder. This ordering is deliberate and is the opposite of the usual arrangement, in which the animal work is done first and the human tissue question is deferred.



The programme is ordered so that the cheapest and most decisive measurement comes first.

Figure 6. Each stage answers a different causal question and each can terminate the programme. Stage 1 asks whether the molecules are present in human disease at all; a negative result there makes the remaining stages moot. Sources: ^{59, 4, 64}.

13.1 Stage 1 — Presence in human disease tissue

Question. Are the two upstream molecules present in human Alzheimer's brain, and is the transducing protein in the membrane?

Design. Post-mortem cortical and hippocampal tissue from neuropathologically confirmed Alzheimer's cases across Braak stages, with age- and sex-matched controls, minimum thirty per group to support stage stratification. Two measurements. First, hCAP-18 and LL-37 by mass spectrometry on microdissected tissue with immunohistochemical localisation, reporting the precursor and the processed peptide separately because the processing step is extracellular and protease-dependent.⁷⁰ Second, CLIC1 quantified as total protein and as the membrane-inserted species, by subcellular fractionation with conformation-selective detection, co-stained with a microglial marker retained in post-mortem tissue.

Controls and confounds. Peripheral blood contamination must be excluded explicitly, since neutrophils are the principal peptide reservoir and agonal state varies. Post-mortem interval must be modelled, since the peptide is protease-generated. Vitamin D status, where recorded, should be carried as a covariate given the primate-specific regulation of the gene.⁷⁶ Because microglial identity genes are lost rapidly outside the tissue environment,¹⁶ this stage is performed in situ and not on isolated cells.

Stopping rule. If neither peptide nor an increased inserted CLIC1 fraction is detectable above control, the upstream arm is not present in the human disease and the programme stops. The remaining stages would then be testing a mechanism that does not occur.

13.2 Stage 2 — Order and dependence in a human cellular system

Question. Does channel insertion precede the degradative deficit, and is the circuit self-maintaining?

Design. Human induced-pluripotent-stem-cell-derived microglia, and the same cells xenografted into a mouse brain environment so that the transcriptional identity is supported rather than lost.²³²⁰³ Three experiments.

Ordering. Apply the peptide at a defined concentration and measure, on a common time base: CLIC1 membrane insertion; oxidative burst; lysosomal pH by ratiometric probe; mitophagy initiation and flux using a tandem pH-sensitive mitochondrial reporter so that initiation and completion are separated; cytosolic mitochondrial DNA by fractionation with quantitative amplification; and interferon-stimulated gene induction. The prediction is a fixed sequence; any observation of the degradative deficit preceding insertion inverts the model.

Pulse and withdrawal. Expose for a defined interval, wash, and follow the same panel for at least four times the clearance half-life of the peptide. Persistence of interferon output and cytosolic mitochondrial DNA after withdrawal supports P8; return to baseline falsifies it. Then interrupt each interior node in turn — CLIC1 by blockade and by knockout, acidification by rescue, cGAS by inhibition — to identify which node must remain engaged for the elevated state to persist. This is the experiment that distinguishes continued initiation from failed termination, and it is the one most often omitted.

Dose-response. The same panel across at least three log units of peptide concentration, reporting amyloid uptake and degradation alongside interferon output, to test P10 and to locate or exclude an interior optimum.

Controls. Citrullinated peptide as a negative control for the DNA-delivery arm,⁷⁷ scrambled peptide for the membrane arm, and a comparison of neutrophil-derived and macrophage-derived peptide given their divergent behaviour.⁷⁸ Anion substitution throughout, to test P3 and to establish whether any readout is chloride-dependent at all.

Stopping rule. If the degradative deficit does not follow channel insertion, or if nothing persists after withdrawal, the circuit is a cascade at best and the closures claimed in Chapter 9 are absent.

13.3 Stage 3 — Necessity in vivo

Question. Is the upstream arm required for the sensing arm in a model carrying both pathologies?

Design. Microglia-specific inducible deletion of *Clic1*, induced after pathology is established rather than constitutively, in a model carrying amyloid and tau. Both sexes in equal numbers and analysed separately, because sex modifies microglial disease trajectories and the interferon arm specifically.¹⁹ Primary outcomes: cytosolic mitochondrial DNA in sorted microglia; interferon-stimulated gene induction; synapse density by a method not dependent on microglial markers; and a cognitive measure with a pre-specified analysis.

Comparators. A microglial *Cgas* deletion arm to establish the position of the two nodes relative to each other,⁸ and a pharmacological arm using a CLIC1 inhibitor with a defined binding residue so that target engagement can be verified by the Arg29 mutant.⁶⁶

Interpretation constraint. A protective effect of microglial *Clic1* deletion is not by itself evidence for this circuit, because microglial manipulations are protective in some designs for reasons unrelated to the pathway.³⁰ The specific claim requires the mechanistic intermediates — cytosolic mitochondrial DNA and interferon induction — to move, not only the endpoint.

Stopping rule. If deletion leaves cytosolic mitochondrial DNA and interferon induction unchanged despite verified deletion, the arms are parallel and the circuit is removed regardless of any cognitive benefit.

13.4 Stage 4 — Reversal, and the junction

Question. Can the state be reversed after establishment, and does the junction behave as claimed?

Design. Two interventions applied after pathology is established, in the same model and with the same outcome panel.

Lysosomal. Restore microglial acidification, using an approach with in vivo precedent,¹²³ and measure the full panel including interleukin-1 β . The falsifying outcome is explicit and plausible: interleukin-1 β rising while interferon falls, which would indicate that re-acidification restores the very permeabilisation step that drives inflammasome activation.¹¹¹⁷⁸ Acidification must be reported as a measured pH distribution rather than as drug administered, because the target is a set-point and overshoot is itself pathological.¹¹³¹¹⁴

Channel. Block CLIC1 after establishment, with pharmacodynamic confirmation by the inserted-species assay developed in Stage 1. The comparison of interest is against sensor-level inhibition: the model predicts that channel blockade reduces interferon output while leaving the cell's response to an exogenous cytosolic DNA challenge intact, whereas STING inhibition removes both. That selectivity, if it exists, is the model's only therapeutic argument, and it also tests whether inhibiting the sensor costs the lysosomal support functions that STING provides.¹⁷⁴¹⁷⁵

Reporting. Stage and sex reported for every outcome; effect sizes with intervals rather than significance alone; and the pre-specified negative outcomes reported with the same prominence as the positive ones.

13.5 What the programme cannot settle

Three limits should be stated in advance. Mouse microglia differ from human microglia in the complement, phagocytic and neurodegeneration modules on which this circuit depends,⁹ so Stage 3 establishes necessity in a model and not in the disease. The vitamin-D-dependent arm of peptide induction cannot be modelled in a mouse at all.⁷⁶ And no stage of this programme establishes that the circuit initiates the disease rather than accompanying it; the strongest available claim from a complete programme would be that the circuit is necessary for a component of ongoing injury at a stage at which a patient could be treated. That is a smaller claim than a causal account of Alzheimer's disease, and it is the one the design supports.

14. Scope, Limitations, and What Would Change the Conclusion

14.1 What this review claims

The claim is bounded: seven elements set down as a list can be arranged into a circuit whose links are individually evidenced, whose order is specified, and whose weakest points are identifiable in advance. It is not a claim that the circuit operates in Alzheimer's disease, because the measurements that would establish that have not been made. That distinction is the whole of the review's value — an unexamined mechanism with a named list of missing experiments is more useful than a plausible one asserted without them.

Three positive findings survive the grading. The mitochondrial DNA–cGAS–STING–interferon arm is well supported, including in human tissue, and is not original here. The peptide–channel pairing is real, published, and largely unabsorbed by the Alzheimer's literature. And the degradative compartment is the only place the two arms can meet, which locates both the model's novelty and its weakness in a single junction.

14.2 What it does not claim

It does not claim that this circuit initiates Alzheimer's disease. Nothing in the evidence base distinguishes initiation from participation, and the human genetic argument, which is the strongest evidence for microglial causality, does not implicate either upstream gene.

It does not claim that microglial activation is uniformly harmful. The evidence that microglial envelopment compacts plaques and insulates neurites,¹⁹¹ that an interferon-linked state can be protective,¹⁹⁵ and that the human neuropathology describes failing rather than overactive cells,²⁶ all argue against that reading.

It does not claim that amyloid deposition is simply pathological. If amyloid- β is an antimicrobial effector,¹⁰⁶¹⁰⁷¹⁰⁸ then a circuit built on a second host-defence peptide is describing a defence system operating at the wrong duration, not a system operating erroneously. That framing changes what a therapy should aim at — duration rather than magnitude — and this review does not have the evidence to decide it.

And it does not claim that any intervention discussed is ready for testing in patients. No cGAS or STING inhibitor has entered a neurodegenerative disease trial, no mitophagy activator has a completed Alzheimer's trial with cognitive endpoints, and the CLIC1 pharmacology, though it includes a licensed drug, has never been directed at this indication.

14.3 The principal limitations of the evidence base

The reliance on rodent and cultured-cell systems is severe and specific. A large part of the CLIC1 microglial literature comes from neonatal rat primary cultures, which lose the transcriptional identity that defines microglia,¹⁶ and rodent and primate microglia diverge in exactly the modules the circuit uses.⁹ The peptide's human induction pathway cannot be modelled in a mouse.⁷⁶

Several load-bearing findings rest on one laboratory. The peptide–channel link has not been replicated in four years. The revision of the CLIC1 mechanism is a single 2025 study, and while it is methodologically strong, the circuit's treatment of what to measure now depends on it.

Cell-type attribution is uncertain in places. Effects assigned to microglia in the oxidative literature have in at least one case been localised to perivascular macrophages,¹⁰ and no clean microglia-conditional NADPH oxidase deletion exists in an amyloid model.

And some of the most directly on-point recent work in the sensing arm exists as preprints rather than peer-reviewed reports. Where such material informed the framing it has been excluded from the grading.

14.4 What would change the conclusion

If Stage 1 finds the peptide and the inserted channel present and elevated in human Alzheimer's brain, the upstream arm moves from unexamined to established-in-principle, and the model's status changes from an arrangement to a hypothesis worth an animal programme.

If the mitophagy signature predicted in P4 is found — initiation preserved or raised, completion blocked, mitochondrial material inside de-acidified autolysosomes — then the junction is real and the circuit's central weakness is repaired.

If the biphasic dose–response of P10 is found, two research programmes that currently contradict each other are reconciled, and the therapeutic question becomes one of restoring an optimum rather than adding or removing a molecule.

Conversely, a monotonic dose–response, an unchanged mitophagy flux at the stage when cytosolic mitochondrial DNA rises, or a microglial *Clic1* deletion that leaves the sensing arm untouched would each remove the model. The first two are the more likely outcomes on present evidence, and the review is written so that they can be recognised quickly rather than argued around.

14.5 A closing remark on method

The elements examined here were assembled before the literature was surveyed, and the survey was conducted with an explicit instruction not to manufacture connections. Of the relationships tested, some were found in the disease, more were found only in other tissues, and ten were not found at all. All three outcomes are reported at the same length and with the same prominence, and the ten absences are printed as a table rather than mentioned in passing.

That is the only methodological commitment this paper makes, and it is the one that determines whether the rest of it is worth anything. A circuit whose missing measurements are listed can be tested. A circuit whose missing measurements are omitted can only be believed.

15. References

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