The Licensing Tyrosine

Above cofilin serine 3 sit two opposed transducers, each activated and condemned by the same phosphorylation — and a disease that misdirects the destruction

Benjamin Aaron Gustafsson

AdultCognitiveDisease.com

August 2026

The direction of change at cofilin serine 3 in Alzheimer's disease is disputed in human tissue and has not been resolved. This paper argues that the sign is not recoverable there, because two antagonistic Rho-family arms write the same mark, and moves one layer up to the tyrosine where the sign is set. The two opposed transducers at that layer — Ephexin5 and Disabled-1 — are built to the same design: the phosphorylation that activates each is the phosphorylation that condemns it, and a single Src-family kinase writes to both. Transience at this control surface is therefore achieved by destruction rather than by dephosphorylation. The paper's original claim is that Alzheimer's synaptic lesion is misdirected proteolysis rather than lost proteolysis; it is untested, and Section X states the measurement that would break it.

Contents

Abstract

Synapse loss is the strongest structural correlate of cognitive impairment in Alzheimer's disease: in the study that established it, neocortical synapse density carried a multivariate correlation of 0.96 with the Dementia Rating Scale, while plaque density contributed only 26 per cent of that model's strength (Terry et al., 1991). A dendritic spine is an actin structure, and the balance between severing and stabilisation of its filaments is set by one protein, cofilin-1, at one residue: **serine 3**.

The direction of change at that residue in Alzheimer's disease is disputed in human tissue, and the dispute has not been resolved. Amyloid-β acting through the immune receptor LilrB2 produces enhanced cofilin signalling — dephosphorylation — reported in mouse and detected in human Alzheimer brain (Kim et al., 2013), a direction independently reproduced with a natural receptor antagonist (Kawaguchi et al., 2022). Amyloid-β acting through Rho-associated kinase *increases* serine-3 phosphorylation in the post-synaptic fraction of human Alzheimer cortex, where that phosphorylation is reported necessary and sufficient for synaptic impairment (Rush et al., 2018). The 2018 paper states that the effect of amyloid on the actin cytoskeleton "remains unknown and contentious." It still is.

This paper does not attempt to adjudicate the sign. It argues that the sign is not recoverable at that residue, and moves one layer up to the place where it is.

The first claim is that phospho-serine-3 is a degenerate mark. Two antagonistic Rho-family arms converge on it. RhoA acting through Rho-associated kinase phosphorylates LIM-domain kinase, and Rac1 and Cdc42 acting through p21-activated kinase phosphorylate the same LIM-domain kinase; both therefore *add* the same phosphate to serine 3 while producing opposite structural outcomes — spine collapse in the first case, spine growth in the second. A measurement of phospho-serine-3 cannot distinguish a collapsing spine from a growing one. Two careful laboratories sampling different synaptic populations will obtain opposite signs, publish both, and be unable to reconcile them, because the quantity they are comparing does not carry the information they need.

The second claim is where that information lives, and it is the paper's structural observation. The two transducers that set the sign — Ephexin5, a Rho-family exchange factor, and Disabled-1, the adaptor of the reelin pathway — are each governed by tyrosine phosphorylation, and each is built to the same unusual design: *the phosphorylation that activates the transducer is the phosphorylation that condemns it*. Phosphorylation of Ephexin5 at tyrosine 361 biases the enzyme toward RhoA and simultaneously recruits the ubiquitin ligase Ube3A, which consigns it to the proteasome (Margolis et al., 2010; Petshow et al., 2025). Reelin-induced tyrosine phosphorylation of Disabled-1 transduces the protective signal and simultaneously targets Disabled-1 for degradation by the ubiquitin–proteasome pathway; the same kinase, Fyn, is required for both, and in its absence the adaptor is not degraded (Arnaud et al., 2003; Bock

and Herz, 2003; Bock et al., 2004). Two opposed arms, converging on one residue, are each self-terminating by *licensed proteolysis*, and a single Src-family kinase writes to both.

The consequence is that **transience at this control surface is not achieved by dephosphorylation. It is achieved by destruction.** A phosphatase resets a mark; it cannot remove a transducer. The spine's capacity to deliver a placed, brief, releasable signal to its actin network depends on a proteolytic clock — the ubiquitin–proteasome system at the transducer, and endosomal sorting, the multivesicular body and the lysosome at the receptor.

The third claim is what Alzheimer's disease does to that clock, and it is not what the field's framing predicts. The disease does not simply lose proteolysis. It *misdirects* it. Amyloid-β binds the fibronectin-repeat domain of EphB2 and triggers the receptor's degradation in the proteasome (Cissé et al., 2011) — so the kinase that licenses the destruction of Ephexin5 is itself destroyed. Downstream, Ephexin5 protein is elevated in the hippocampi of human Alzheimer patients (Sell et al., 2017). In the same neuron, in the same compartment, one proteolytic arm over-runs and the other under-runs. The lesion is not too much destruction, and not too little. It is the wrong substrate.

That reading is supported by the position of the endosomal abnormality in the disease's timetable. Enlargement of the early endosome is the earliest known disease-specific structural change in sporadic Alzheimer's disease: present in neocortical pyramidal neurons at preclinical stages, present in Down syndrome from 28 weeks of gestation, accentuated by *APOE* ε4, and — decisively — *absent* in advanced familial disease caused by presenilin mutation, which establishes that it is not a consequence of amyloid deposition (Cataldo et al., 2000). Loss-of-function variants in *SORL1*, the gene for the sorting receptor that delivers cargo to the retrieval machinery, are causal for Alzheimer's disease, and in human neurons haploinsufficiency enlarges endosomes while complete loss adds lysosomal and autophagic failure (Hung et al., 2021). Apolipoprotein E4 sequesters the reelin receptor ApoER2 in intracellular compartments and reduces its surface expression, impairing the receptor's ability to signal (Chen et al., 2010) — and that block is reversible by inhibiting the endosomal proton leak channel NHE6, which restores both receptor recycling and reelin's modulation of excitatory synapses (Xian et al., 2018). Peroxidation of the lipid cargo generates reactive aldehydes that crosslink ApoE covalently to the ligand-binding modules of ApoER2 (Ramsden et al., 2022) — a lesion no sorting step can undo, because the ligand and its receptor have become one molecule.

The prediction. If the sign is set above the serine and read out degenerately at it, then the two residues should *decouple* in disease. The measurement is a paired one and has never been made: the phospho-tyrosine-361 fraction of Ephexin5 and the phospho-serine-3 fraction of cofilin, quantified in the same synapses of human cortex, with active-RhoA and active-Cdc42 determined in the same tissue. In control cortex the two marks should covary, because the tyrosine governs the serine. In Alzheimer cortex the covariance should fall while the dispersion of the serine mark rises — the serine being driven by chronic ligand occupancy that the tyrosine no longer gates. This fails cleanly: if the two residues covary in disease as in control, the sign *is*

recoverable at the serine, and the account offered here is wrong.

What it forbids. It forbids lowering Ephexin5 as a therapeutic strategy: heterozygous loss-of-function mutations in *ARHGEF15*, the gene encoding it, cause autosomal-dominant cerebral small-vessel disease with osteoporotic fracture, acting through RhoA/ROCK2 *inactivation* (Ding et al., 2023) — the proposed therapy is a phenocopy of a human dementing vasculopathy. It forbids total-protein readouts of any transducer whose abundance and activity are two readings of one covalent mark. It forbids inducing autophagy in a neuron that cannot yet acidify. And it forbids the assumption that a kinase inhibitor can substitute for a protease: a drug that lowers a phosphorylation level does not remove the protein that the phosphorylation was supposed to remove.

Scope, stated at the outset. This is a convergence claim about the *execution* of synaptic loss, not a claim about what initiates the disease. It does not explain who gets Alzheimer's disease, why particular neurons fail first, or the substantial fraction of dementia not attributable to the pathologies conventionally measured. Section XIII states the boundary in full, and Section XIV states what would refute the argument.

I. What kind of claim this is, and what it is not

Claims about Alzheimer's disease fail more often for being of the wrong *kind* than for being unsupported. A claim about what initiates the disease requires evidence from the earliest affected people, which for a disorder with a two-decade preclinical phase is largely inaccessible; that inaccessibility is a sufficient explanation for why so many initiation claims coexist and so few are settled. A claim about what the disease *converges on* requires evidence that multiple upstream causes arrive at one place, and is adjudicated by intervention in both directions.

This paper makes a convergence claim, and a narrow one. It says that however Alzheimer's disease begins — and it plainly begins in more than one way — the route by which an upstream cause becomes a lost dendritic spine passes through a small control surface above the actin cytoskeleton; that the surface is governed by proteolysis rather than by phosphatase activity; and that the disease damages the proteolytic governance in a specific and asymmetric way that has not been named.

Three restrictions are honoured throughout, and it is worth stating them before the argument rather than after it.

It does not explain who gets the disease. Nothing here addresses initiation, selective vulnerability, or why one person with amyloid becomes demented and another does not.

It applies to the synaptic-execution arm. A substantial fraction of dementia is not attributable to the pathologies usually measured, and a substantial fraction of people meeting neuropathological criteria are not demented. Section XIII states what portion of the disease is claimed and what portion is not.

Its central positive proposal is untested. The structural observation in Sections IV and V — that the two opposed transducers share a self-terminating design — is verifiable directly from the published record and is not in doubt. The inference drawn from it in Section IX, that Alzheimer's synaptic lesion is misdirected rather than lost proteolysis, is an inference. It is graded as such in the ledger of Section XV, and Section XIV states what would break it.

One further remark on method. The argument below repeatedly turns on the difference between a *level* and an *object*. Much of the molecular literature on this disease reports levels: how much of a protein, how much of a phosphorylation. Almost every mechanism described here is instead about whether a particular molecule is still present in a particular compartment. Those are

different measurements, they can move in opposite directions, and several of the field's live contradictions dissolve when the distinction is enforced.

II. Why the serine cannot report its own cause

2.1 The residue, and what is established about it

A dendritic spine is a bag of actin. Its volume — and therefore the strength of the synapse it carries — is the running balance of filament nucleation and elongation against filament severing and turnover.

Severing is performed by cofilin, the major actin-depolymerising factor of mammalian neurons, and cofilin is switched by phosphorylation on serine 3. Phosphorylated cofilin cannot bind filamentous actin and the network is stable; dephosphorylated cofilin binds and severs. LIM-domain kinases add the phosphate. The slingshot phosphatases and chronophin remove it (Bamburg et al., 2021; Kang and Woo, 2019).

Three properties make this the natural residue at which to look for a mechanical lesion. It is a convergence point by construction, because every receptor system that alters spine structure must eventually alter the actin network and the great majority do so through the Rho-family GTPases. It is directly implicated in this disease in human tissue: inclusions containing cofilin and actin are prominent in hippocampal and cortical neurites of post-mortem Alzheimer brain and are most prominent in neurites contacting amyloid deposits (Minamide et al., 2000), and these rods are present in Alzheimer brain and not in normal brain (Bamburg and Bernstein, 2016). And it is druggable now: Rho-kinase inhibitors exist, one is clinically available, and the class is under active investigation for this indication (Zheng et al., 2025).

2.2 Two antagonistic arms write the same mark

Here is the structural fact on which this paper's first claim rests, and it is standard cell biology rather than a new finding — which is precisely why its consequence for the Alzheimer literature has gone unstated.

The Rho-family GTPases that control spine actin do not form one pathway. They form two, with opposite structural outputs.

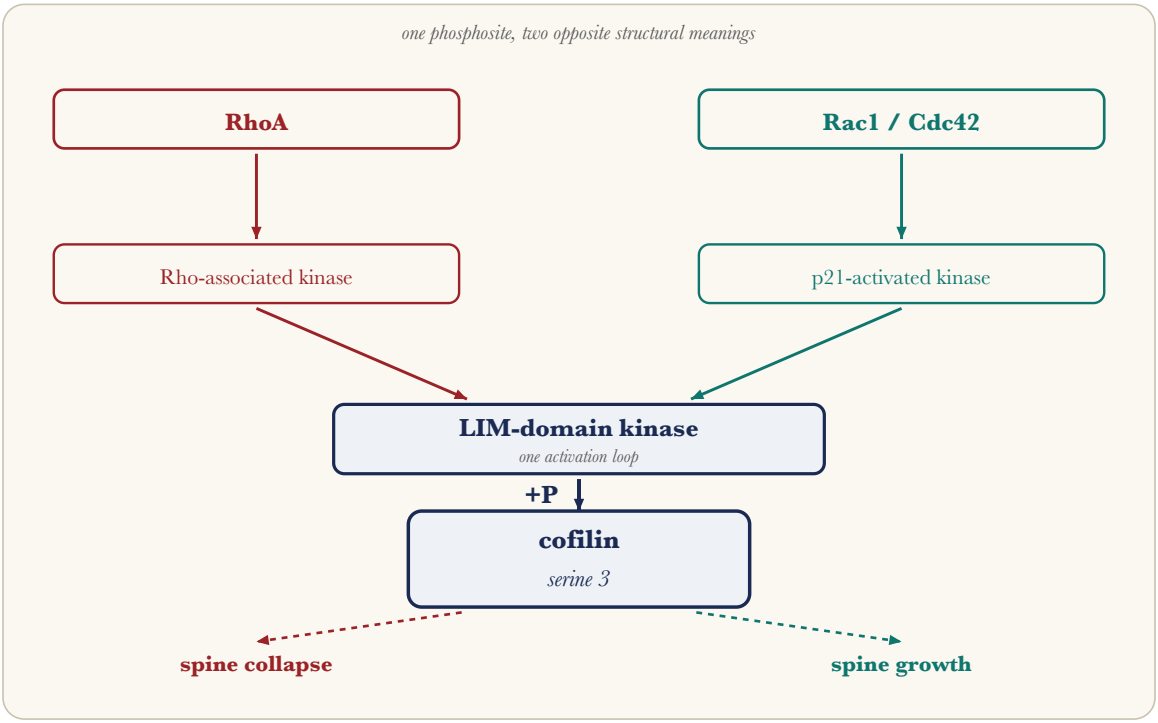
The contractile arm. RhoA activates Rho-associated kinase. Rho-associated kinase phosphorylates LIM-domain kinase on its activation-loop threonine. LIM-domain kinase phosphorylates cofilin at serine 3. The structural output is spine shrinkage and collapse.

The protrusive arm. Rac1 and Cdc42 activate p21-activated kinase. p21-activated kinase phosphorylates the *same* LIM-domain kinase on the *same* activation-loop threonine. LIM-domain kinase phosphorylates cofilin at serine 3. The structural output is spine growth and stabilisation.

Both arms raise phospho-serine-3. They mean opposite things.

This is not a subtlety about kinetics or subcellular pools. It is a many-to-one collapse in the information carried by the mark. A spine that is collapsing under RhoA tone and a spine that is growing under Cdc42 tone can present the same phospho-serine-3 signal, and no antibody against that phosphosite can tell them apart. The mark is *degenerate*: it is the shared output of two pathways whose difference is the thing the observer wants to know.

Figure 1 · Two antagonistic arms write the same mark



RhoA acting through Rho-associated kinase and Rac1/Cdc42 acting through p21-activated kinase converge on the same activation loop of the same LIM-domain kinase, and therefore add the same phosphate to the same residue — while producing opposite structural outcomes. A measurement of phospho-serine-3 cannot distinguish a collapsing spine from a growing one. The degeneracy is in the variable, not in the sampling: even a perfect single-synapse measurement would not recover the sign. This is the most economical explanation of why careful laboratories report opposite directions from human Alzheimer tissue and cannot reconcile them.

Three consequences follow immediately, and they matter for how the existing human data should be read.

First, a bulk phospho-cofilin measurement is uninterpretable in principle, not merely in practice. The usual objection to homogenate measurements is that they average across heterogeneous compartments. That objection is correct and it is not the point being made here. Even a *perfectly compartment-resolved* measurement of phospho-serine-3, made at a single synapse with no averaging at all, would still not say which arm produced it. The degeneracy is in the variable, not in the sampling.

Second, the published contradiction is what this architecture predicts. One group reports amyloid lowering phospho-cofilin through an immune receptor in human Alzheimer brain (Kim et al., 2013), reproduced independently with a receptor antagonist (Kawaguchi et al., 2022). Another reports amyloid *raising* phospho-cofilin through Rho-kinase in a post-synaptic-enriched fraction of human Alzheimer cortex, with fasudil rescuing the phenotype (Rush et al., 2018). If phospho-serine-3 is the shared output of a collapsing arm, a growing arm and a receptor-driven phosphatase arm, then which sign a given preparation returns is a function of which arm dominates the material sampled. Both results can be correct measurements of the same disease.

Third — and this is the constructive move — the information the field wants is not lost. It has simply been sought at the wrong residue. The two arms differ upstream, at the level of which exchange factor is active on which GTPase. That is where the sign is set, and Section IV shows that in the relevant transducer it is set at a single tyrosine.

2.3 An observation about the phosphatase limb, which is also captured

It might be objected that the dephosphorylating limb is unitary and therefore informative even if the kinase limb is not. It is not unitary, and it is also engaged by the disease.

Cofilin is dephosphorylated by the slingshot phosphatases and by chronophin, and the regulation of slingshot homolog 1 in this disease has been worked out in some detail. Amyloid-β signalling converges on slingshot-mediated cofilin activation; the scaffolding protein RanBP9 positively regulates slingshot levels and mediates amyloid-induced translocation of cofilin to mitochondria and the induction of cofilin–actin pathology in cultured cells, primary neurons and in vivo, with RanBP9 reduction protecting against cofilin–actin pathology, synaptic damage and gliosis in an amyloid model (Woo et al., 2015). A review of the wider regulation lists LIM-domain kinase 1 and slingshot homolog 1 as the two nodes on which amyloid-driven signalling impinges, together with β-arrestin, RanBP9, chronophin, phospholipase D1 and 14-3-3 (Kang and Woo, 2019).

So the residue has at least four regulatory inputs of two opposite signs, and the disease engages both limbs. Whatever else this is, it is not a system whose state can be summarised by one number.

2.4 Which cell, and why it must be named

Cofilin is ubiquitous and Alzheimer's disease is not, so a claim at this residue is worth nothing until it names a cell. The literatures assembled below converge, without any of them saying so, on the **excitatory glutamatergic pyramidal neuron of neocortex and hippocampus, at its dendritic spines**.

The Rho-kinase measurement is made in a post-synaptic-density-enriched synaptosome fraction and its functional readout is the insertion of the AMPA-receptor subunit GluA1 — a glutamatergic synapse by definition (Rush et al., 2018). The immune-receptor arm is anchored at excitatory synapses of human cerebral cortex, where the receptor and its complement ligand colocalise (Brott et al., 2025), with plasticity deficits in hippocampal long-term potentiation and visual-cortical ocular dominance (Kim et al., 2013). The Ephexin5 arm is characterised in hippocampal pyramidal neurons and in the dentate gyrus (Margolis et al., 2010; Sell et al., 2017; Hamilton et al., 2017). The reelin arm terminates on the same cell: Disabled-1, the adaptor through which the entire reelin signal is transduced, is expressed predominantly in pyramidal neurons (Pesold et al., 1999). And the receptor that carries the protective ligand, ApoER2, is concentrated in entorhinal layer II stellate neurons, the prosubiculum–CA1 border and scattered neocortical pyramids — a distribution that follows the map of early neurofibrillary pathology, while its close relative VLDLR, sharing the same ligand and the same adaptor, is expressed ubiquitously including in the layer 4 stellate cells that resist tangles (Ramsden et al., 2022).

This convergence on one cell is a result of reading the preparations side by side rather than a premise of the argument, and it is what makes them commensurable at all.

It also fixes what this paper is not about. The parvalbumin-positive fast-spiking interneuron — the cortex's most conspicuously vulnerable inhibitory element — is characteristically aspiny or sparsely spiny and receives excitatory input on shafts and soma rather than on spines. The mechanics described here are the mechanics of a spine and do not transfer to it directly. No claim is made that they do.

III. The dispute at serine 3, stated from the primary record

Before moving above the residue it is necessary to set out, in their own terms, what the disputing laboratories actually measured. The gradings used here and in the ledger of Section XV follow a five-point maturity scale: **M1** human, population-scale or replicated across independent cohorts; **M2** human, single cohort or tissue series; **M3** animal, replicated; **M4** animal, single laboratory; **M5** in vitro or inference from adjacent findings.

3.1 Amyloid removes the phosphate, through an immune receptor

Kim and colleagues reported that murine PirB and its human orthologue LilrB2, present in human brain, are receptors for soluble amyloid-β oligomers at nanomolar affinity; that the first two extracellular immunoglobulin domains mediate the interaction; and that engagement leads to enhanced cofilin signalling, also seen in human Alzheimer brains. In mice the deleterious effect of amyloid oligomers on hippocampal long-term potentiation required PirB, and in a transgenic model PirB contributed to adult memory deficits and mediated loss of synaptic plasticity in juvenile visual cortex (Kim et al., 2013). Enhanced cofilin signalling means more active cofilin, which means less phosphate at serine 3.

The same laboratory has since shown that C4d, a complement cleavage product of previously unknown function, binds LilrB2 and PirB with nanomolar affinity; that C4d and LilrB2 colocalise at excitatory synapses in human cerebral cortex and with amyloid-β in Alzheimer's disease; that both C4 and C4d increase with age and more so in Alzheimer's; and that infusing C4d into wild-type mouse cortex significantly reduces dendritic spine density, with the loss completely prevented by knockout of PirB (Brott et al., 2025).

Direction: phosphorylation down. Consequence: destabilisation. Maturity M3 for the mechanism, M2 for the human colocalisation and elevation.

3.2 An independent laboratory reproduces the dephosphorylation

Kawaguchi and colleagues, working on an unrelated problem — an endogenous antagonist of the Nogo receptor and PirB called LOTUS — showed that LOTUS inhibits amyloid-β binding to PirB; that in cultured hippocampal neurons from LOTUS-overexpressing transgenic mice, amyloid-induced *dephosphorylation* of cofilin and amyloid-induced loss of PSD-95 were both suppressed; that the amyloid-induced fall in dendritic spine density was improved; and that human LOTUS inhibits amyloid binding to human LilrB2 in the same way (Kawaguchi et al., 2022). A different laboratory, a different country and a different tool — a natural competitive antagonist rather than a knockout — reproduced the direction.

Direction: phosphorylation down. Independent of the originating group. Maturity M4.

3.3 Amyloid adds the phosphate, in human Alzheimer cortex

Rush and colleagues, with no shared authorship with the above, reported elevated phospho-cofilin-1 in the post-synaptic-enriched fraction of synaptosomes from cortical samples of APP/PS1 mice and of human Alzheimer cases. In primary cortical neurons, amyloid-β oligomers induced rapid actin *stabilisation* and *increased* phospho-cofilin within thirty minutes. Fluorescence recovery after photobleaching and calcium imaging in neurons expressing active or inactive cofilin mutants indicated that cofilin phosphorylation is necessary and sufficient for amyloid-induced synaptic impairment via actin stabilisation, occurring *before* the formation of cofilin–actin rods. The clinically available Rho-kinase inhibitor fasudil prevented the actin stabilisation, the synaptic impairment and the synaptic loss by blocking cofilin phosphorylation; amyloid also blocked potentiation-induced insertion of GluA1 in a fasudil-sensitive manner (Rush et al., 2018).

The paper opens by stating that how amyloid-β affects the actin cytoskeleton "remains unknown and contentious," and notes that others have described increased cofilin phosphorylation in Alzheimer patients. It does not cite or address the opposite finding from the receptor literature.

Direction: phosphorylation up. Consequence: stabilisation. Maturity M2 for the human measurement.

3.4 And the rods require the phosphate to be absent

Minamide and colleagues showed that mediators of neurodegeneration induce rod-like inclusions of cofilin and actin in axons and dendrites; that rods form spontaneously in neurons overexpressing *active* cofilin, indicating that activation by dephosphorylation is sufficient to induce them; and that persistent rods disrupt microtubules and degenerate the distal neurite without killing the neuron (Minamide et al., 2000). Bamburg and colleagues established the biochemistry: cofilin binds cooperatively along ADP-actin subunits and severs filaments at low cofilin-to-actin ratios while stabilising them at high ratios; rod formation requires oxidation of cofilin to disulfide-linked dimers; and rods sequester cofilin, block transport and exacerbate mitochondrial membrane-potential loss (Bamburg and Bernstein, 2016; Bamburg et al., 2021).

> *Direction: requires phosphorylation down. Maturity M2 for rods in human Alzheimer brain, M4 for the induction mechanism.*

3.5 The shape of the problem

	writes to Ser3	direction	actin result	human data	source
Aβ → LilrB2/PirB	yes	**dephosphorylate**	destabilise	yes, AD brain	Kim 2013
Aβ → PirB (antagonist test)	yes	**dephosphorylate**	destabilise	human LilrB2 binding	Kawaguchi 2022
Aβ → ROCK → LIM kinase	yes	**phosphorylate**	stabilise	yes, AD cortex	Rush 2018
Reelin → ApoER2/Dab1	yes	**phosphorylate**	stabilise	no	Chai 2009
Cofilin–actin rods	requires low pSer3	**dephosphorylate**	bundle, block transport	yes, AD brain	Minamide 2000

Rows one and three are the same ligand, the same residue, the same disease and opposite signs, each supported by measurement in human Alzheimer tissue. Row four is a *protective* input that writes in the same direction as row three, which is the second reason the mark cannot be read: an elevated phospho-serine-3 signal is equally consistent with pathological Rho-kinase tone and with an intact reelin brake.

That last point deserves emphasis, because it is not a subtlety. In a tissue where both the destructive contractile arm and the protective reelin arm add the same phosphate, a rise in phospho-cofilin is compatible with the disease mechanism running *and* with the protective mechanism working. No inference about therapy can be drawn from the level. The remainder of this paper is about where the difference is written down.

IV. The licensing tyrosine, first instance: Ephexin5 at Y361

4.1 The architecture as originally described

Excitatory synapse formation in the developing brain is not merely promoted; it is actively restrained, and the restraint must be lifted at the right time and place.

Ephexin5, the product of *ARHGEF15*, was identified in 2010 as a RhoA guanine-nucleotide exchange factor expressed highly in immature neurons that negatively regulates excitatory synapse development until ephrin-B binding to the EphB receptor tyrosine kinase triggers its phosphorylation, ubiquitination and degradation. The ligase that performs the tagging is Ube3A — the gene deleted or mutated in Angelman syndrome and duplicated in some autism-spectrum disorders. Degradation of Ephexin5 promotes EphB-dependent excitatory synapse development (Margolis et al., 2010).

The chain is worth stating in order, because everything below turns on it:

EphB2 signalling → phosphorylation of Ephexin5 on a tyrosine within a conserved regulatory motif → recruitment of Ube3A → ubiquitination → proteasomal destruction → removal of the restraint → synaptogenesis proceeds.

Note what kind of mechanism this is. The synapse is not permitted to form because a brake has been *switched off*. It is permitted to form because a brake has been *removed from the cell*. The signal is terminated by proteolysis, and the phosphorylation is the licence.

4.2 The revision: a switch, not a brake

The proposition that Ephexin5 is a RhoA-selective exchange factor and a simple brake was placed under strain in 2017 and substantially revised in 2025.

Hamilton and colleagues found that reducing Ephexin5 increased spine outgrowth and increasing it decreased outgrowth, consistent with a brake — but also that Ephexin5-GFP was elevated on the dendritic shaft at the sites of future new spines *before* those spines appeared, and that lowering Ephexin5 *inhibited* new spine outgrowth in response both to global activity increases and to local glutamatergic stimulation. They concluded that Ephexin5 serves a dual role: a brake on overall spine outgrowth, and a necessary component of the site-specific formation of new spines. The same paper reported that increased neural activity produced a **proteasome-dependent reduction in**

the levels of Ephexin5 in neuronal dendrites (Hamilton et al., 2017).

That last observation is the one to hold. The controlled variable is dendritic Ephexin5; the control is proteolytic; the trigger is activity.

The mechanism of the duality was established in 2025. Ephexin5 activates both RhoA and Cdc42, and in knockout brain the activated pools of both GTPases fall substantially. Live imaging of Förster-resonance-energy-transfer GTPase biosensors at single spines showed that during plasticity induced by high-frequency glutamate uncaging, Ephexin5 regulates the activation of Cdc42 but *not* of RhoA. The selectivity of Ephexin5 for Cdc42 activation is regulated by tyrosine phosphorylation, which is itself regulated by neuronal activity; phospho-tyrosine Ephexin5 falls developmentally, and chemical long-term potentiation reduces it sharply within minutes by dephosphorylation rather than degradation, since the fall survives proteasome inhibition. A phosphorylation-dead mutant at the key tyrosine, Y361F, enhances Cdc42 activation while leaving RhoA activation unchanged. Functionally, spines in Ephexin5-knockout neurons failed to sustain activity-driven growth; re-expression of full-length Ephexin5 restored growth while a catalytically dead exchange factor did not, and the Y361F phosphomutant rescued growth in knockout neurons. Downstream of neuronal activity, Ephexin5 *positively* regulates synaptic growth and stabilisation (Petshow et al., 2025).

Ephexin5 is therefore not a brake that the adult brain has switched off. It is a switch that the adult brain keeps and throws, and the throwing is what learning-associated spine growth depends on. Substrate selection is a property not of the enzyme but of its phosphorylation state.

4.3 The coincidence that has not been drawn out

The residue at which the switch is thrown is the same residue whose phosphorylation was characterised in 2010 as the event that recruits Ube3A and consigns Ephexin5 to the proteasome (Margolis et al., 2010; Petshow et al., 2025).

Phosphorylation at that tyrosine does two things at once. It biases the exchange factor toward RhoA — the collapse arm — and it marks the exchange factor for destruction. Dephosphorylation likewise does two things at once. It releases the Cdc42-directed activity — the growth arm — and it protects the protein from ubiquitin-dependent turnover.

Abundance and activity are therefore not independent variables in this system. They are two readings of one covalent modification, and this has consequences that no intervention on the pathway can avoid.

It makes "Ephexin5 is elevated" ambiguous on its own. A cell with elevated Ephexin5 may have lost the kinase input that marks it for destruction, in which case the accumulated protein is hypo-phosphorylated at the tyrosine and, on the 2025 model, Cdc42-biased and growth-directed.

Or it may have gained a phosphorylation input not coupled to Ube3A recruitment, in which case the accumulated protein is RhoA-biased and collapse-directed. These two states have opposite structural consequences for the spine. Current measurements do not distinguish them, because the antibodies and assays used to report Ephexin5 levels report total protein.

It makes the tyrosine, not the serine below it, the informative residue. Phospho-tyrosine-361 selects RhoA over Cdc42; RhoA and Cdc42 are precisely the two arms that Section II showed converge indistinguishably on phospho-serine-3. The mark that disambiguates the degenerate one sits two steps upstream, and it has never been measured in human Alzheimer tissue.

4.4 The sign problem in the disease, and the missing kinase

The disease model built on this architecture holds that amyloid-β re-activates the developmental programme by stripping the receptor that licenses the destruction. Amyloid-β acutely promotes Ephexin5 production in mature hippocampal neurons and in hAPP mice; Ephexin5 expression is elevated in the hippocampi of human Alzheimer patients; genetic removal of Ephexin5 from hAPP mice eliminated hippocampal dendritic spine loss and rescued behavioural deficits; and shRNA-mediated reduction in the dentate gyrus of presymptomatic adolescent hAPP mice was sufficient to protect against later cognitive impairment (Sell et al., 2017). That is a loss-of-function rescue with a prevention arm, which is a stronger design than most in this field.

The first step of that model is that amyloid depletes EphB2, and it is well supported: amyloid-β oligomers bind the fibronectin-repeat domain of EphB2 and trigger the receptor's degradation in the proteasome; knockdown of EphB2 reduced NMDA-receptor currents and impaired long-term potentiation in the dentate gyrus of non-transgenic mice, while increasing EphB2 expression in the dentate gyrus of hAPP transgenic mice reversed deficits in NMDA-receptor-dependent potentiation and memory (Cissé et al., 2011).

But the model as stated contains a tension in its sign, and the tension is instructive rather than fatal. EphB2 is the kinase that phosphorylates the licensing tyrosine. Loss of that kinase should *lower* the phosphorylated fraction — which explains the accumulation, since the phospho-form is the degraded form, but which predicts, on the 2025 substrate-switching model, a Cdc42-biased and therefore growth-promoting pool. The observed phenotype is spine loss. Either something restores the RhoA bias that the loss of EphB2 removes, or the sign of the model is wrong.

There are candidates for that something, and they are testable. Protein kinase C epsilon phosphorylates and activates Ephexin5 in immature neurons in a manner that suppresses spines. Src-family kinases are the other obvious class, and Fyn in particular is mislocalised to the post-synaptic density in a tau-dependent manner in Alzheimer models (Ittner et al., 2010), placing

an active tyrosine kinase in exactly the compartment in question — a point developed in Section VI. If a tyrosine kinase other than EphB2 phosphorylates the licensing residue *without* licensing Ube3A recruitment, because the ligase requires a co-incident EphB2-dependent signal the disease has removed, then the Alzheimer neuron would hold a pool of Ephexin5 that is simultaneously abundant and RhoA-locked.

That is the state the disease model requires and the state nobody has measured. It is also the state this paper predicts, for a reason that becomes clear once the second transducer is examined: a system whose OFF switch is a protease will, when the protease is misdirected, come to rest in exactly this configuration — the effector present, the licence unwritten, the activity ungated.

4.5 A natural experiment in chronic loss, and what it forbids

In 2023 the gene encoding Ephexin5 acquired a Mendelian human phenotype, and it is the most therapeutically consequential fact on this pathway.

ARHGEF15 was identified as a causal gene for autosomal-dominant hereditary cerebral small-vessel disease. A heterozygous non-synonymous mutation co-segregated completely in two families; a further non-synonymous mutation and a stop-gain mutation were found in two sporadic cases. Every mutation carrier also had severe osteoporosis, and some had osteoporotic fractures. In vitro, the mutations produced RhoA/ROCK2 **inactivation** with consequent F-actin disorganisation in vascular smooth-muscle and endothelial cells, and osteoblast dysfunction through inhibition of Wnt/β-catenin signalling. A transgenic mouse carrying one of the variants developed small-vessel-disease pathology and behavioural phenotypes with severe osteoporosis. The authors' conclusion is explicit: these are loss-of-function mutations, and loss of function causes the disease (Ding et al., 2023).

Cerebral small-vessel disease is itself a leading cause of vascular dementia and of both ischaemic and haemorrhagic stroke. The therapeutic strategy the synaptic model originally implied — chronic reduction of Ephexin5 function — now has a human phenotype attached to it, and the phenotype is a dementing cerebrovascular disease with skeletal fragility. One functional copy is not enough in the adult human.

The direction is worth dwelling on. In neurons the Alzheimer proposal casts RhoA activation by Ephexin5 as the destructive event and its suppression as therapeutic. In vessels and bone, loss of function causes disease *through RhoA/ROCK2 inactivation*. There is nothing incoherent in this — Rho-family signalling is context-specific, and a set-point too high in a spine may be load-bearing in a smooth-muscle cell. But it converts the therapeutic problem from one of delivery into one of dose and direction simultaneously, and it removes the argument that made the target attractive: that a protein nearly absent from healthy adult tissue could be inhibited with impunity.

Two limits on that inference should be stated. The vascular finding is evidence about what happens when Ephexin5 function is lost; it bears on the therapy, not on the pathophysiology, and it is not evidence that Ephexin5 fails to participate in Alzheimer's disease. And the in vivo Alzheimer evidence rests on amyloid-precursor-protein overexpressing mice from one laboratory; because such lines overproduce the full complement of precursor fragments, a phenotype that appears in an overexpressing line and has not been sought in a knock-in line carries an unquantified probability of being a model artefact. Whether Ephexin5 is elevated in humanised *App* knock-in mice, and whether its deletion rescues spine loss there, is not addressed in the published literature we could identify. It is a cheap experiment and an obvious one.

4.6 Angelman syndrome as the reciprocal experiment

Loss of Ube3A should stabilise Ephexin5, and if Ephexin5 were simply a RhoA-directed brake the predicted consequence would be severe spine loss. That is not the observed consequence in Angelman models.

The substrate-switching model resolves this without special pleading: in the developing brain, where phospho-tyrosine Ephexin5 is a shrinking fraction and activity is high, accumulated Ephexin5 is Cdc42-biased and therefore stabilising rather than destructive. The resolution is satisfying and it is also a warning. It demonstrates that in a real human disorder, a large increase in Ephexin5 protein produced a phenotype opposite in sign to the one the brake model predicts. The Alzheimer model asks us to believe that a comparable increase in a different cellular context produces the predicted sign. That may well be true. It is not, at present, shown, and Angelman syndrome establishes that the inference from abundance to sign is not safe.

Which is the general lesson of this section, and it applies with equal force to the second transducer: **in a system where one covalent mark sets both what an enzyme does and how long it survives, no measurement of how much of the enzyme is present can tell you what the enzyme is doing.**

V. The licensing tyrosine, second instance: Disabled-1

5.1 The protective arm and its wiring

The opposing input to spine actin in this cell is reelin, and its architecture is the mirror image of the one just described.

Reelin binds directly and specifically to the ectodomains of the very-low-density lipoprotein receptor and ApoER2, and blockade of those receptors abolishes reelin-induced tyrosine phosphorylation of Disabled-1 in cultured primary embryonic neurons. Mice lacking either reelin or both receptors show hyperphosphorylation of tau (Hiesberger et al., 1999). The downstream chain runs from tyrosine-phosphorylated Disabled-1 to phosphatidylinositol-3-kinase through its regulatory subunit P85α, to Akt, and thence to inhibition of glycogen synthase kinase-3β — a principal tau kinase.

The actin limb of the same signal was characterised a decade later. Reelin signalling leads to serine-3 phosphorylation of n-cofilin, which renders it unable to depolymerise filamentous actin and thereby stabilises the cytoskeleton; the chain runs through ApoER2, Disabled-1, Src-family kinases and phosphatidylinositol-3-kinase. Phosphorylation was localised to the leading processes of migrating neurons as they approached the reelin-containing marginal zone (Chai et al., 2009).

Two features of this arm are load-bearing for what follows.

One adaptor, two ledgers. Disabled-1 is the single point through which the whole reelin signal passes, and it has at least two outputs of first-rank importance in this disease: stabilisation of actin at cofilin serine 3, and restraint of glycogen synthase kinase-3β and therefore of tau phosphorylation. Anything that removes Disabled-1 from the compartment removes both at once. This is the most economical available explanation for why a receptor lesion should produce a *cytoskeletal* and a *tau* phenotype simultaneously, without either being downstream of the other.

The reader is not the writer. In adult cortex reelin is expressed primarily by GABAergic interneurons and secreted extrasynaptically into the surrounding matrix, but only by a defined subset: interneurons expressing neuropeptide Y or somatostatin are reelin-positive and parvalbumin-expressing basket and chandelier cells are never reelin-positive, while Disabled-1 is expressed predominantly in pyramidal neurons (Pesold et al., 1999). The protective signal is written by one interneuron class into a shared extracellular compartment and read by a different, excitatory

cell. The residues in dispute are all on the reader.

5.2 The same design: the phosphorylation that transduces is the phosphorylation that condemns

Here is the finding that makes this paper's structural observation more than an analogy.

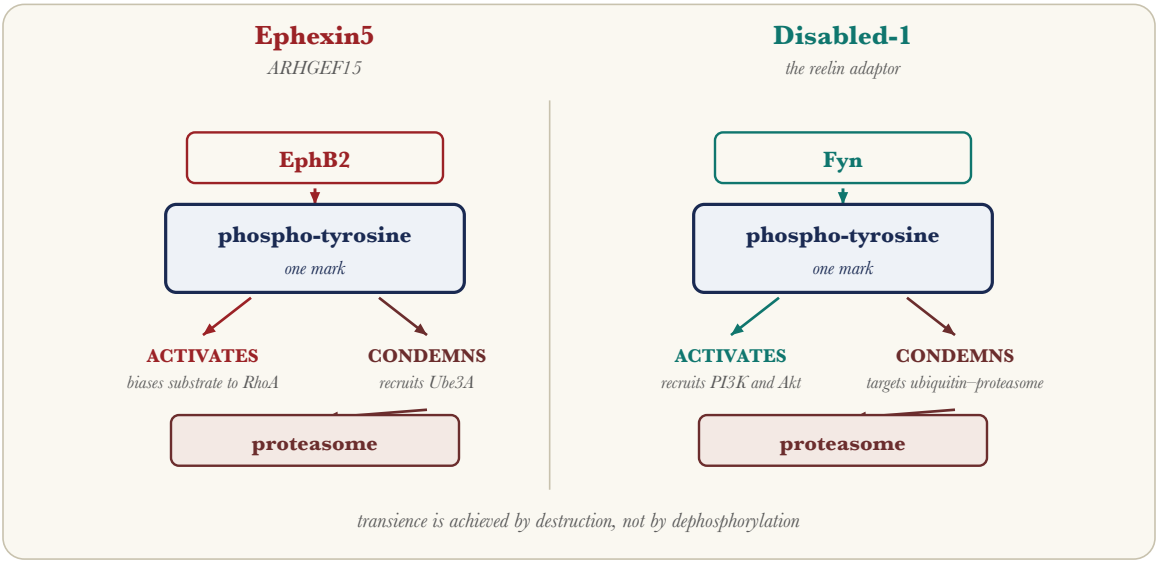
Reelin treatment targets Disabled-1 for proteolytic degradation by the ubiquitin–proteasome pathway. Tyrosine phosphorylation of Disabled-1 — but not phosphatidylinositol-3-kinase activation — is required for that proteasomal targeting. Genetic deficiency in the Disabled-1 kinase Fyn prevents the degradation. The reelin-induced degradation depends on ApoER2 and the very-low-density lipoprotein receptor in a gene-dose-dependent manner. And pharmacological blockade of the proteasome prevents the formation of a proper cortical plate in slice culture, so the degradation step is not an epiphenomenon of signalling but a required part of it (Bock et al., 2004).

The upstream kinase requirement was established in the same period from two directions. Reelin activates Src-family kinases in neurons in a manner dependent on ApoER2, the very-low-density lipoprotein receptor and Disabled-1; Disabled-1 is both a substrate and an activator of those kinases; and Disabled-1 protein levels *rise* in Fyn-deficient mice, which is the signature of impaired reelin signalling (Bock and Herz, 2003). Independently, reelin-induced Disabled-1 tyrosine phosphorylation was shown to be blocked by Src-family but not Abl-family inhibitors, and Fyn was found to be required for proper Disabled-1 levels and phosphorylation in vivo and in vitro, with Src becoming important when Fyn copy number is reduced. Reelin activates Fyn to phosphorylate *and downregulate* Disabled-1 (Arnaud et al., 2003).

Set the two transducers side by side.

	Ephexin5	**Disabled-1**
role at the spine	selects RhoA (collapse) or Cdc42 (growth)	transduces reelin: stabilises actin, restrains GSK-3β
governing mark	phospho-tyrosine 361	phospho-tyrosine (multiple sites)
kinase writing it	EphB2; other tyrosine kinases uncharacterised	Fyn, with Src partially redundant
what the mark does #1	biases substrate toward RhoA	recruits PI3K/Akt, transduces the signal
what the mark does #2	recruits Ube3A → proteasome	targets the adaptor → ubiquitin–proteasome
termination mechanism	destruction of the transducer	destruction of the transducer
level rises when	the licensing kinase is lost	reelin signalling is impaired, or Fyn is absent

Figure 2 · The licensing tyrosine: the mark that activates is the mark that condemns



The two opposed transducers above cofilin serine 3 are built to the same unusual design. Phosphorylation of Ephexin5 at tyrosine 361 biases the exchange factor toward RhoA and recruits the ubiquitin ligase Ube3A that consigns it to the proteasome. Reelin-induced tyrosine phosphorylation of Disabled-1 transduces the protective signal and targets the adaptor for ubiquitin–proteasome degradation; Fyn is required for both, and in its absence the adaptor is not degraded. Abundance and activity are therefore not independent variables in either arm — they are two readings of one covalent mark, which is why a total-protein measurement cannot say what the transducer is doing.

The two opposed arms above cofilin serine 3 are built to the same design. In each, one tyrosine phosphorylation simultaneously activates the transducer and licenses its destruction. In each, the accumulation of the transducer is the biochemical signature of a *failed* signal rather than an amplified one. In each, transience is achieved by proteolysis and not by dephosphorylation.

We are not aware of this parallel having been stated. It is verifiable directly from the four sources tabulated above, and it is the observation on which the rest of this paper is built.

5.3 Why the design makes sense, and what it costs

A cell that needs to deliver a *placed* and *brief* structural instruction to its cytoskeleton has a problem that a simple kinase–phosphatase pair does not solve well. A phosphatase resets the level of a mark, but the transducer remains in the compartment, available to be re-activated by any subsequent input, including noise. If the instruction must be local — this spine, not its neighbour — and must not persist, then removing the transducer is the more reliable termination. It is also self-limiting in a useful way: the more strongly the signal is transduced, the more completely the transducer is consumed, so the response is intrinsically pulse-like and cannot run away.

The price is a specific fragility, and it is the disease's opportunity. A system that terminates by proteolysis has its temporal control vested in the proteolytic machinery, not in the signalling

machinery. Its OFF is not a chemical reaction that runs at the same rate regardless of cellular state; it is a *service*, provided by the ubiquitin–proteasome system, by endosomal sorting, and ultimately by the lysosome. Anything that degrades that service degrades the cell's capacity to make brief signals, without touching a single kinase or receptor.

That is the hinge of this paper. It is why the next section asks what the disease does to the service rather than to the signal.

VI. Fyn: one kinase, both ledgers

Fyn deserves a section of its own, because it is where the two arms touch and because it is the most frequently proposed drug target in this region of the map.

6.1 What Fyn does on the protective side

Fyn is the physiological Disabled-1 kinase. It is required for proper Disabled-1 phosphorylation and levels in vivo; its absence raises Disabled-1 protein, the signature of an unsignalled pathway; and its absence prevents the reelin-induced proteasomal degradation of Disabled-1 (Arnaud et al., 2003; Bock and Herz, 2003; Bock et al., 2004). Fyn is thus not merely a transducer of the protective arm; it is the enzyme that writes the licence terminating it. Without Fyn, the reelin brake cannot be applied *and* cannot be released.

6.2 What Fyn does on the destructive side

Fyn is also the principal intracellular transducer of amyloid toxicity at the post-synaptic density, by two convergent routes.

Soluble amyloid-β oligomers bind cellular prion protein with high affinity, and at the post-synaptic density extracellular oligomers bound to lipid-anchored prion protein activate intracellular Fyn to disrupt synapses. The coupling requires metabotropic glutamate receptor 5: only its co-expression allowed prion-protein-bound oligomers to activate Fyn. Prion protein and mGluR5 interact physically, cytoplasmic Fyn forms a complex with mGluR5, and signalling through the complex mediates eEF2 phosphorylation and dendritic spine loss; mGluR5 antagonism reversed deficits in learning, memory and synapse density in familial-Alzheimer transgenic mice (Um et al., 2013).

Separately, tau has a dendritic function in the post-synaptic targeting of Fyn. Missorting of tau in mice expressing truncated tau, and the absence of tau in tau-null mice, both disrupt post-synaptic targeting of Fyn; this uncouples NMDA-receptor-mediated excitotoxicity and mitigates amyloid toxicity, and tau deficiency prevented memory deficits and improved survival in an amyloid-forming model. The deficits were also fully rescued by a peptide uncoupling the Fyn-mediated interaction of the NMDA receptor with PSD-95 (Ittner et al., 2010).

So in the Alzheimer neuron, tau delivers Fyn to the compartment and amyloid activates it there.

6.3 The consequence, which is uncomfortable for a target

Put the two ledgers together. **The kinase that the disease over-activates at the post-synaptic density is the same kinase that the protective reelin pathway requires in order to be applied and terminated.**

This has three implications, and none of them is that Fyn is a good target.

Global Fyn inhibition is predicted to be sign-ambiguous, for the same reason phospho-serine-3 is. Inhibiting Fyn removes the amyloid-driven excitotoxic transduction, which should help. It also impairs Disabled-1 phosphorylation, which weakens the reelin brake on glycogen synthase kinase-3β and on tau, and blocks the proteasomal clearance of Disabled-1, which degrades the pulse structure of the protective signal. The net effect depends on the relative weight of the two arms in the tissue being treated, which is exactly the quantity nobody measures.

Fyn is the leading candidate for the missing kinase of Section 4.4. The disease model requires that something restores RhoA bias to Ephexin5 after amyloid has stripped EphB2. Fyn is a tyrosine kinase, it is mislocalised to the post-synaptic density in this disease by a tau-dependent mechanism, and it is active there. If Fyn phosphorylates the licensing tyrosine without licensing Ube3A recruitment — because the ligase requires a co-incident EphB2-dependent signal the disease has removed — then the Alzheimer dendrite holds an Ephexin5 pool that is abundant, RhoA-biased, and not scheduled for destruction. This is a specific, cheap, falsifiable hypothesis: it predicts that Fyn inhibition in an amyloid-bearing neuron will lower phospho-tyrosine-361 without lowering total Ephexin5, and will shift active-RhoA down and active-Cdc42 up. It is stated here as a hypothesis and graded as inference in Section XV.

And it explains why the same molecule can be reported as protective and destructive without either report being wrong. Fyn is not a node with a sign. It is a shared enzyme serving two opposed clients, and the clients differ in whether the disease has left their receptors intact.

VII. Termination is proteolytic, and a phosphatase cannot substitute

7.1 The distinction that organises this section

The literature on phosphorylation in Alzheimer's disease is overwhelmingly a literature of *levels*: how much phospho-tau, how much phospho-cofilin, how much phosphatase activity. The mechanisms in Sections IV and V are not about levels. They are about whether a particular protein is still in a particular compartment.

The distinction is not pedantic, and one example makes it concrete. Suppose the phosphatase acting on the licensing tyrosine were fully intact and the ligase were absent. The mark would be reset normally, and a measurement of phospho-tyrosine would look unremarkable — but the transducer would accumulate with every cycle, because the mark's *other* function, condemning the protein, was never executed. The level says nothing. The census says everything.

7.2 The phosphatases of this system, and why they are the wrong tool

The relevant phosphatases are not idle in this disease, and their state is worth recording precisely because it does not rescue the argument.

Protein phosphatase 2A is the principal tau-dephosphorylating enzyme in brain, acting as a trimer of catalytic, scaffolding and regulatory subunits. Multiple abnormalities have been reported in Alzheimer's disease: decreased protein levels of the scaffolding and B55α regulatory subunits, reduced catalytic-subunit methylation at Leu309 attributed to impaired methyltransferase function, increased catalytic-subunit phosphorylation at Tyr307, up-regulation of the endogenous inhibitors I1 and I2, and loss of enzymatic activity — with the caveat, stated by the reviewers themselves, that the individual alterations have not been uniformly replicated and converge on the enzyme from different directions (Torrent and Ferrer, 2012).

On the cofilin limb, the resetting phosphatases are slingshot and chronophin, and slingshot homolog 1 is positively regulated by RanBP9, which mediates amyloid-induced translocation of cofilin to mitochondria and the induction of cofilin–actin pathology; reducing RanBP9 protected against that pathology, against synaptic damage and against gliosis in an amyloid model (Woo et al., 2015; Kang and Woo, 2019).

Two conclusions follow, and they pull in the same direction.

The phosphatase limb is itself engaged by the disease, so it cannot be treated as a stable reference against which kinase-driven changes are measured.

More importantly, a phosphatase cannot do the job that the proteasome does here. Restoring protein phosphatase 2A activity would lower phospho-tau and would lower the phosphorylated fraction of a licensing tyrosine. It would not remove an accumulated exchange factor from a dendrite. Dephosphorylating Ephexin5 would, on the 2025 model, *protect it from turnover* while releasing its Cdc42-directed activity — a therapeutic move whose sign depends on which arm the tissue needs. This is the practical form of the paper's claim: at a control surface terminated by destruction, phosphatase-directed therapy addresses the mark and leaves the object.

7.3 The three proteolytic services of the dendrite

Three distinguishable proteolytic systems act on the molecules in this account, and it is useful to keep them separate because the disease affects them differently.

The canonical ubiquitin–proteasome system. This is the route characterised for both transducers: Ube3A-dependent ubiquitination of Ephexin5 (Margolis et al., 2010) and ubiquitin–proteasome-dependent degradation of Disabled-1 (Bock et al., 2004). It is also the route by which amyloid disposes of EphB2 (Cissé et al., 2011). Note that this last point makes the system a participant in the disease rather than merely a casualty of it.

A dendritic, activity-regulated membrane proteasome. Neurons possess a neuronal membrane proteasome that degrades intracellular proteins into peptides released directly into the extracellular space; neuronal stimulation promotes its activity and the production of those peptides, which then act as endogenous and selective activators of NMDA receptors, driving calcium influx, sustained CREB phosphorylation and immediate-early gene expression (Türker et al., 2024). Its properties — dendritic, activity-regulated, specialised for recently made protein — coincide closely with the properties of the reported activity-dependent, proteasome-dependent fall in dendritic Ephexin5 (Hamilton et al., 2017). Whether the second degrades the first has not been tested, and we state it as a hypothesis rather than a finding; the characterised Ephexin5 route is Ube3A-dependent and therefore canonical, but a dendrite contains both machines and a protein may have more than one disposal route with different kinetics and different triggers.

The endosomal–lysosomal system. This handles the receptor limb rather than the cytosolic transducers, and it is the subject of the next section. Internalised receptors are sorted at the early endosome either for retrieval and re-use or for delivery through the multivesicular body to the lysosome for destruction. Whether a receptor can transduce a *second* signal is decided here.

The unifying observation is that the spine's control surface has no OFF switch that is not a protease. Its kinases are shared, its phosphatases are non-specific and disease-engaged, and the only mechanism that reliably ends a local structural instruction is the removal of the molecule that

carried it.

VIII. The receptor limb: sorting decides whether a signal can be re-sent

8.1 The earliest lesion in the disease is a sorting lesion

If the argument above is right, then the state of the endosomal system should matter to synaptic signalling from very early in the disease. It does, and the evidence is unusually clean.

Enlargement of the early endosome is the earliest known disease-specific structural abnormality in Alzheimer's disease. Cataldo and colleagues found enlarged rab5-positive early endosomes in neocortical pyramidal neurons at preclinical stages, when Alzheimer-like neuropathology was still restricted to the entorhinal region; elevated rab4 and translocation of rabaptin-5 to endosomes, implying that both endocytic uptake and recycling were activated; enlargement in Down syndrome pyramidal neurons as early as 28 weeks of gestation, decades before classical neuropathology; minimal influence of normal ageing and of the other neurodegenerative diseases examined; accentuation of the enlargement by inheritance of the *APOE* ε4 allele at preclinical stages; and — the control that makes the timing argument work — **normal endosome size at advanced stages of familial disease caused by presenilin-1 or presenilin-2 mutation**, establishing that the abnormality is not a consequence of amyloid deposition (Cataldo et al., 2000).

That last result is worth pausing on. In the form of the disease with the highest lifetime amyloid burden, the endosomal abnormality is absent. Whatever produces it in sporadic disease, it is not the peptide.

8.2 SORL1: the sorting receptor, and a causal human gene

The cargo receptor that delivers material to the retrieval machinery is SORLA, encoded by *SORL1*, and it is one of the few genes in this field whose human genetics carries the direction of causation without a model organism.

In human neurons, a *SORL1* truncating mutation produces haploinsufficiency and enlarged endosomes. Analysis of isogenic wild-type, heterozygous and homozygous-null neurons showed that haploinsufficiency alone produces endosome dysfunction, while complete loss adds defects in lysosomal function and autophagy. Critically, the endolysosomal dysfunction caused by loss of SORLA was relieved by antisense-oligonucleotide-mediated reduction of amyloid precursor protein, demonstrating that presenilin-1, the precursor protein and SORLA act in a common pathway regulating the endolysosomal system (Hung et al., 2021).

The same pathway is not confined to neurons. In human induced-pluripotent-stem-cell-derived microglia, loss of SORLA decreases lysosomal degradation and lysosomal enzyme activity through altered trafficking of lysosomal enzymes; phagocytic uptake of fibrillar amyloid-β and of synaptosomes is *increased* while degradation is reduced, so both substrates accumulate aberrantly in lysosomes, and lysosomal exocytosis is also impaired (Mishra et al., 2025).

That microglial result is relevant here for a reason beyond completeness. Synaptosomes are taken up and not digested. The cell tasked with clearing synaptic material acquires the same lesion as the cell whose synapses are being lost, which means the disposal failure is not a property of one cell type but of a shared machine.

8.3 ApoER2 recycling, APOE4, and the reversal experiment

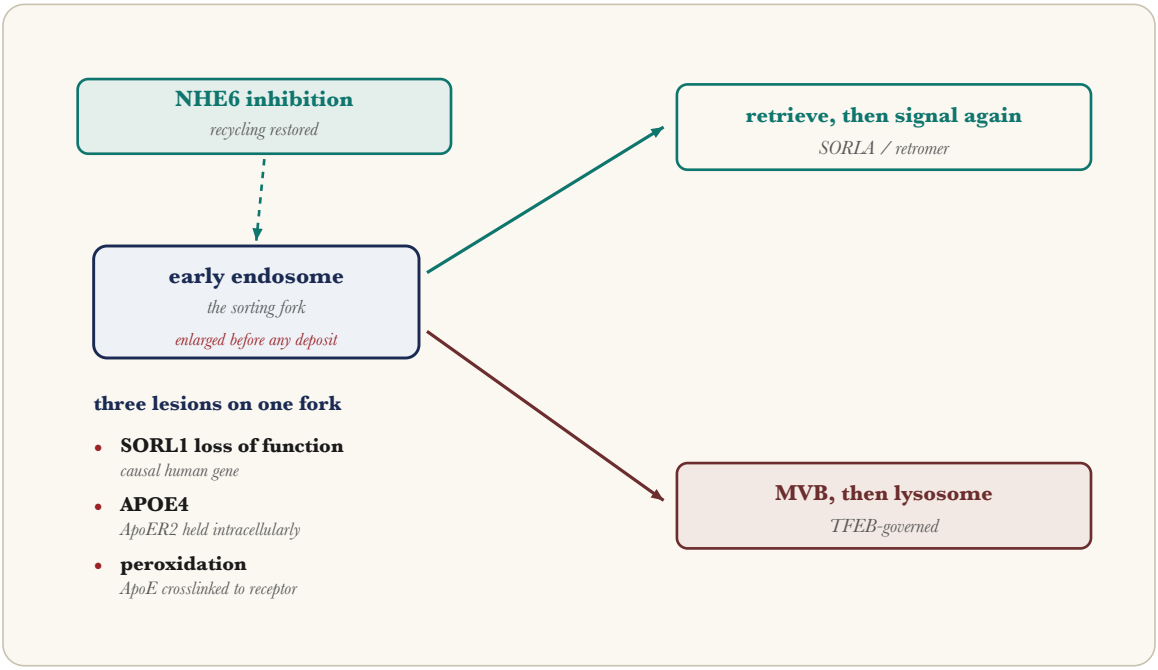
The protective arm's receptor is subject to the same logic, and here the evidence is interventional in both directions.

Apolipoprotein E4 selectively impairs synaptic plasticity and NMDA-receptor phosphorylation by reelin. It reduces neuronal surface expression of ApoER2 — the dual-function receptor for both ApoE and reelin — as well as of NMDA and AMPA receptors, by **sequestration in intracellular compartments**, thereby critically reducing reelin's ability to enhance synaptic glutamate-receptor activity. As a result, reelin's capacity to prevent long-term-potentiation suppression by extracts of Alzheimer brain is severely impaired in slices from knock-in mice expressing the human ApoE4 isoform (Chen et al., 2010).

Read that mechanism carefully. The receptor is not absent, mutated or blocked by a competing ligand. It is *in the wrong compartment*. The signal fails for a trafficking reason.

And it is reversible for a trafficking reason. Pharmacological and genetic inhibition of NHE6, the primary proton-leak channel of the early endosome, completely reverses the ApoE4-induced recycling block of ApoER2 and of the AMPA- and NMDA-type glutamate receptors that are co-endocytosed in complex with it, and restores the reelin-mediated modulation of excitatory synapses that ApoE4 impairs (Xian et al., 2018).

Figure 3 · The sorting fork, its three lesions, and the one repair



Whether a receptor can transduce a second signal is decided at the early endosome. Enlargement of that compartment is the earliest known disease-specific structural change: present before deposition, present in Down syndrome from 28 weeks of gestation, accentuated by APOE ε4 — and absent in advanced presenilin-mutation familial disease, which establishes that it is not a consequence of amyloid. Three separate lesions act on this fork. Only one intervention in the cited literature repairs it: inhibiting the endosomal proton-leak channel NHE6 restores ApoER2 recycling and with it reelin's modulation of excitatory synapses — adding no signal and blocking no pathway.

This is, so far as we can determine, **the only published intervention in this region of the map that restores the capacity to signal rather than adding or subtracting a signal.** It does not supply reelin, block amyloid, inhibit a kinase or activate a phosphatase. It repairs the compartment, and the signalling returns. If the argument of this paper is right, that is the category of intervention that should work, and its existence is the strongest available support for the general claim.

8.4 Peroxidation: a lesion no sorting step can undo

There is one failure mode at this receptor that trafficking repair cannot address, and it belongs in the account because it defines the boundary of the therapeutic proposal.

Lipoprotein receptors of the LDL-receptor family bind their ligands through acidic, calcium-coordinating ligand-binding modules, and the ligands bind through basic, lysine-enriched recognition motifs. Both ApoE and reelin engage ApoER2 through cationic, lysine-rich motifs. Lysine is precisely the residue that reactive lipid aldehydes attack.

Ramsden and colleagues showed that ApoE and ApoER2 peptides and proteins are susceptible to attack by reactive lipid aldehydes, generating lipid–protein adducts and **crosslinked ApoE–ApoER2 complexes**. In post-mortem specimens from twenty-six individuals across the clinical range, ApoER2 was strongly expressed in the terminal zones of the entorhinal–hippocampal perforant-path projections; ApoE, lipid-aldehyde-modified ApoE, reelin, ApoER2, Disabled-1 and the downstream markers Thr19-phosphorylated PSD95 and Thr508-phosphorylated LIM-domain kinase 1 accumulated near neuritic plaques in those terminal zones; and several pathway markers were higher in Alzheimer cases and correlated positively with histological progression and inversely with cognitive performance (Ramsden et al., 2022).

Two features of this deserve emphasis for the present argument.

The accumulating species is a signalling pathway, not a waste product. Reelin, Disabled-1 and phosphorylated LIM-domain kinase 1 accumulate together in the vulnerable zones. On the reading developed here, that is what a failure of *termination* looks like: the transducers of the protective arm are present in excess in exactly the compartment where the arm has stopped working — which is the same signature that elevated Ephexin5 carries on the opposite arm, and the same signature that raised Disabled-1 carries in the Fyn-deficient mouse (Bock and Herz, 2003).

And a covalent crosslink is outside the reach of the clock. A receptor sequestered in an endosome can be returned to the surface by fixing the pH. A receptor covalently joined to its ligand cannot be separated by any sorting step, because ligand and receptor have become one molecule. This defines the limit of the repair strategy proposed in Section XVI and marks the point at which prevention replaces repair. It should be recorded that this chemistry has been demonstrated with purified peptides and recombinant protein at aldehyde concentrations chosen to drive the reaction, and that no published work has isolated a crosslinked ApoE–ApoER2 species from human brain and identified it by mass spectrometry. The human evidence is immunohistochemical and is graded accordingly.

8.5 The multivesicular body, and what is found inside it

If receptors condemned to destruction pass through the multivesicular body, then the state of that compartment matters, and there is a human observation about it that has been available for more than two decades.

Amyloid-β42 localises predominantly to multivesicular bodies of neurons in normal mouse, rat and human brain. In transgenic mice and in human Alzheimer brain, intraneuronal amyloid-β42 increases with age and accumulates in multivesicular bodies within presynaptic and *especially postsynaptic* compartments, and that accumulation is associated with abnormal synaptic morphology **before** amyloid-plaque pathology (Takahashi et al., 2002).

The compartment through which the spent receptor must pass in order to be destroyed is the compartment in which the pinning ligand accumulates, in the post-synaptic terminal, before plaques. Whatever else is true, the disposal route and the toxic species share an address.

8.6 TFEB, the lysosome, and a feed-forward loop

The terminal step of the system is under transcriptional control, and its failure closes a loop.

Transcription factor EB, the master regulator of lysosomal biogenesis, has an essential role in the lysosomal exocytosis of selected tau species; its loss of function significantly reduced interstitial-fluid tau in mice expressing mutant tau and in conditioned media from mutant-tau-expressing primary neurons, and the reduction was associated with **enhanced intraneuronal pathology and accelerated spreading** — establishing that this route is a clearance mechanism whose loss worsens the disease (Xu et al., 2021).

And the substrate impairs the regulator. Expression of a tauopathy-associated carboxy-terminal fragment of tau leads to lipid accumulation in cell lines and primary cortical neurons through a block of autophagic clearance and lysosomal degradative capacity; on autophagy induction, the fragment inhibited nuclear translocation of transcription factor EB, and cells and neurons expressing it also showed changes in endosomal protein expression (Pollack et al., 2024).

So the cargo disables the machine that clears the cargo. Once a threshold is crossed, the disposal clock does not merely run slow; it runs progressively slower as a function of what it has failed to clear. This is the mechanism by which a graded, age-related decline in clearance capacity can become a staged disease.

8.7 The sugar bed, which is also a degradative substrate

One further element of the protective arm belongs here, because it is usually discussed as extracellular architecture rather than as cargo.

Reelin cannot activate its receptor as a simple two-body ligand. N-sulfated heparan sulfate is an obligate co-receptor for reelin-induced ApoER2 dimerisation: full-length reelin binds heparan sulfate at 17 ± 5 nM, tightened to 10 ± 2 nM by the resilience-associated COLBOS variant; N-sulfation is the critical glycan determinant; heparinase treatment or knockout of the N-sulfation enzyme NDST1 strips reelin from the cell surface; and heparinase or free heparin in the medium reduces reelin-induced ApoER2 dimerisation while N-desulfated heparin does not (Pan et al., 2025).

Two consequences follow, and the second is a drug-level conflict.

The staging depot is itself turned over. Heparan-sulfate proteoglycans are internalised and degraded lysosomally. A neuron whose lysosomal function is failing does not merely accumulate cargo; it alters the sulfated surface on which the protective ligand is staged. The link is

mechanistically plausible and, so far as we can determine, unmeasured in this disease; it is graded as inference and stated as an experiment in Section XI.

And a therapy aimed at tau propagation would silence the reelin brake by the same chemistry. Heparan-sulfate proteoglycans are the route by which pathological tau enters neurons, which has made heparin mimetics an attractive strategy for blocking transcellular tau spread. A heparin mimetic given for that purpose would, by the identical binding chemistry demonstrated above, block reelin-induced ApoER2 dimerisation. The conflict is verified at drug level and argues that the sulfated matrix must be modulated rather than blocked.

A conflation should also be disposed of, since it recurs in this area. The perineuronal net is a **chondroitin**-sulfate structure; reelin's requirement is for **heparan** sulfate, a component of the diffuse matrix that surrounds all central nervous tissue rather than of the condensed net (Fawcett et al., 2022). Reelin therefore does not require a perineuronal net in order to signal, and the two can be separated experimentally with a pair of enzymes.

IX. Misdirected proteolysis: the disease's actual signature

9.1 The two facts, placed side by side

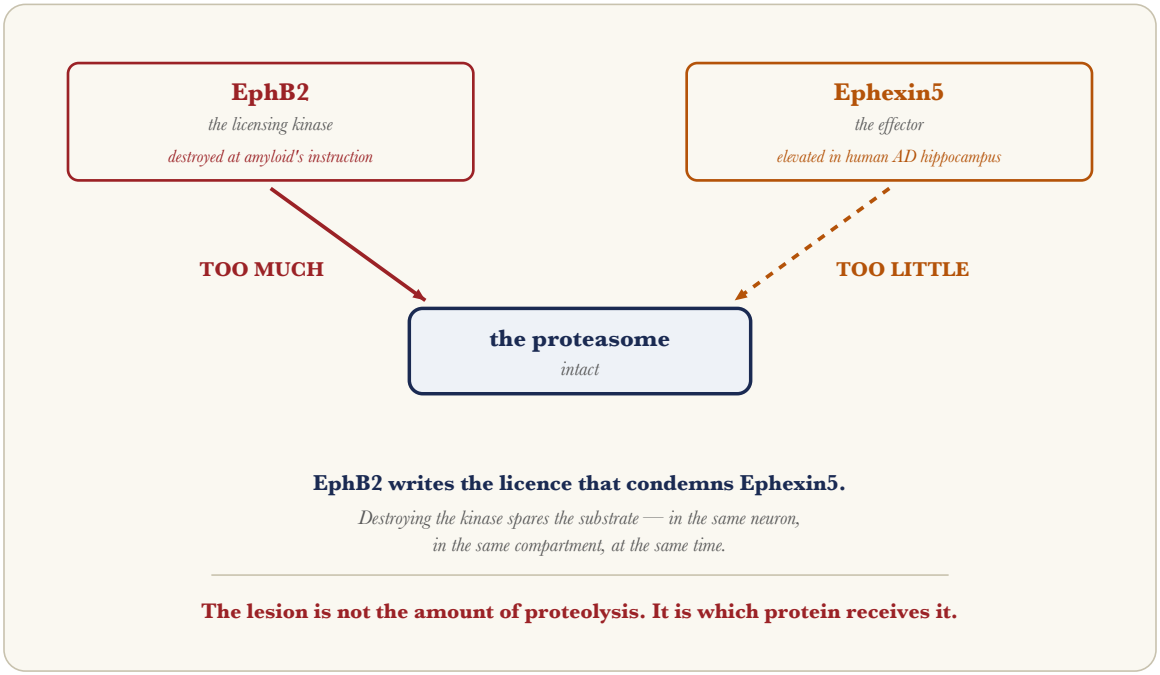
The argument now reduces to two published findings about the same pathway in the same cell type, which have not to our knowledge been set against each other.

The licence-writing receptor is destroyed too much. Amyloid-β oligomers bind the fibronectin-repeat domain of EphB2 and trigger its degradation *in the proteasome*, and EphB2 depletion is critical in amyloid-induced neuronal dysfunction: knockdown reduced NMDA-receptor currents and impaired dentate potentiation in non-transgenic mice, while restoring EphB2 in amyloid-bearing mice reversed potentiation and memory deficits (Cissé et al., 2011).

The effector is destroyed too little. Ephexin5 expression is elevated in the hippocampi of human Alzheimer patients and in the hippocampi of hAPP mice; amyloid acutely promotes its production; and its genetic removal eliminates spine loss and rescues behaviour in the model (Sell et al., 2017).

These are not independent observations that happen to concern the same pathway. They are two ends of one chain. EphB2 is the kinase whose signalling phosphorylates the licensing tyrosine and thereby recruits Ube3A to destroy Ephexin5 (Margolis et al., 2010). Amyloid feeds that kinase to the proteasome. The proteasome then does not receive the substrate whose destruction the kinase was supposed to authorise.

Figure 4 · Misdirected proteolysis: one machine, two errors of opposite sign



Amyloid-β binds the fibronectin-repeat domain of EphB2 and triggers the receptor's degradation in the proteasome. EphB2 is the kinase whose signalling writes the licence that condemns Ephexin5. Downstream, Ephexin5 protein is elevated in the hippocampi of human Alzheimer patients. In one neuron and one compartment, one arm of ubiquitin-dependent degradation over-runs while the arm it was supposed to license under-runs. Any account with a single scalar called clearance predicts that the two move together; they move apart.

The proteolytic system is not failing. It is executing the wrong instruction. In the same neuron, in the same compartment, one arm of ubiquitin-dependent degradation over-runs — removing a receptor the cell needs — while the arm it was supposed to license under-runs, leaving in place an exchange factor the cell was supposed to clear.

9.2 Why this is a better description than "loss of clearance"

The dominant framing of proteostasis in this disease is a deficit framing: clearance capacity declines with age, substrates accumulate, and the disease is what accumulation produces. That framing is well supported for bulk cargo and it is not being disputed. It is, however, insufficient here, for three reasons.

A pure capacity deficit does not predict the EphB2 result. If proteolysis were simply failing, a receptor targeted for proteasomal degradation would be *spared*, not lost. The observed direction is the opposite. Amyloid's action at EphB2 is a gain of degradation, achieved by presenting a substrate to an intact machine.

A pure capacity deficit does not predict the sign asymmetry. The two transducers in this account respond to a proteolytic failure in the same direction — both accumulate — while the receptor responds in the opposite direction. Any account with a single scalar variable called "clearance" predicts that all three move together. They do not.

And a pure capacity deficit gives the wrong therapeutic instruction. If the problem were capacity, then increasing degradative flux should help across the board. It does not follow on the present account, and Section XII states the specific case in which it is predicted to harm.

The more accurate description is that the disease alters the *specificity* of proteolysis in this compartment. That is a different kind of lesion, and it is one that a measurement of total proteasome activity, total autophagic flux or total lysosomal enzyme content cannot detect.

9.3 The pinning ligand is itself a proteolytic product

One more element completes the picture, and it is an irony worth stating plainly rather than leaving implicit.

C4d is a complement cleavage product. It is made by proteolysis. It binds LilrB2 and PirB at nanomolar affinity, colocalises with LilrB2 at excitatory synapses in human cerebral cortex and with amyloid-β in Alzheimer's disease, rises with age and more so in Alzheimer's, and is sufficient to strip spines when infused into wild-type mouse cortex — an effect completely prevented by knockout of PirB (Brott et al., 2025).

So a proteolytic fragment supplies the chronic occupancy at one receptor while a proteolytic failure leaves the transducer of the opposing arm in place. Two proteolytic systems, opposite signs, one synapse. Whatever this disease is doing to protein destruction, "less of it" does not describe it.

9.4 The claim, stated so that it can be attacked

Alzheimer's synaptic execution is a lesion of proteolytic specificity in the dendritic compartment, read out at a phosphosite that cannot report it.

The control surface above cofilin serine 3 is made transient by destruction rather than by dephosphorylation. In disease, the licensing receptor of one arm is degraded by an intact proteasome at the instruction of amyloid, while the effector it was supposed to condemn accumulates; the receptor of the opposing arm is sequestered in the wrong compartment by the commonest genetic risk factor, or covalently joined to its ligand by peroxidation; and the sorting and lysosomal machinery that would resolve either problem is impaired from before the appearance of any deposit, by a lesion that human genetics shows to be causal.

The observable consequence at the residue below is a mark that is driven by chronic ligand occupancy rather than gated by the tyrosine above it. That is a measurable prediction, and Section

X states it.

X. What this predicts

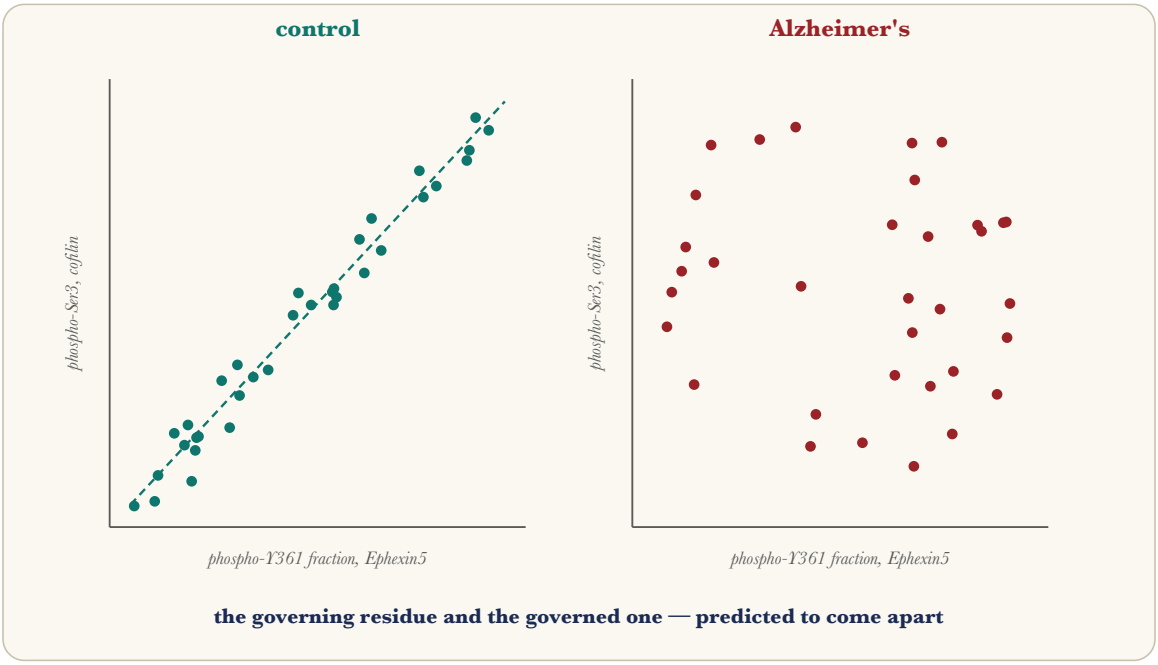
10.1 The primary prediction: the two residues decouple

In a healthy spine, phospho-serine-3 is downstream of the GTPase state, and the GTPase state is set at the licensing tyrosine. The two marks should therefore covary: changes in the tyrosine's phosphorylated fraction should be accompanied by predictable changes in the serine's, because the upper residue governs the lower one.

If this paper is right, that governance fails in disease — not because the serine stops being phosphorylated, but because it starts being driven from elsewhere: by chronic ligand occupancy at LilrB2, by contractile tone from an unlicensed exchange factor, by a reelin arm whose receptor is in the wrong compartment.

The prediction: in Alzheimer cortex the covariance between the phospho-tyrosine-361 fraction of Ephexin5 and the phospho-serine-3 fraction of cofilin, measured across individual excitatory synapses, is reduced relative to age-matched control — while the dispersion of the serine mark rises.

Figure 5 · The prediction: the governing residue and the governed one come apart



In healthy cortex the tyrosine above sets the GTPase state, which sets the serine below, so the two marks should covary across individual synapses. If the disease drives the serine from elsewhere — chronic ligand occupancy at LilrB2, contractile tone from an unlicensed exchange factor, a reelin arm whose receptor is in the wrong compartment — then the covariance should fall while the dispersion of the serine mark rises. No account currently in the literature predicts this; each predicts a mean shift at one residue and is silent about the relationship between residues. It fails cleanly: if the two marks covary in disease as in control, the sign is recoverable where the field has been looking, and this paper is wrong.

This is a strong prediction in the useful sense. It is not implied by any account currently in the literature: each of the existing accounts predicts a mean shift at one residue and is silent about the relationship between residues. It is quantitative. And it fails cleanly — if the two marks covary in disease as in control, then the sign *is* recoverable at the serine, the degeneracy argument of Section II has no consequence in real tissue, and the paper's central proposal is wrong.

Two secondary predictions sharpen it.

Total Ephexin5 should be a poor predictor of active RhoA, and the phospho-tyrosine fraction a good one. This is the direct test of Section 4.3, and it discriminates the two states — hypo-phosphorylated and Cdc42-biased versus phosphorylated and RhoA-biased — that current total-protein measurements cannot separate. If total Ephexin5 predicts RhoA activity as well as the phosphorylated fraction does, the licensing architecture has no consequence in disease tissue.

The dispersion of the serine mark should be bimodal and receptor-associated, with a LilrB2-associated dephosphorylated mode and a contractile phosphorylated mode;

co-staining for LilrB2 assigns each synapse to a mode. If the modes do not separate on receptor content, the compartment argument fails even if the dispersion result holds.

10.2 The genotype prediction

If the neuron's disposal capacity sets the resting level of the effector, then the commonest genetic risk factor should order that level.

The prediction: dendritic Ephexin5, measured as total protein and as phospho-tyrosine-361 fraction, is highest in ApoE4 and lowest in ApoE2 neurons, before any amyloid challenge. The knock-in neurons required already exist. A negative result — no genotype ordering — removes the mechanistic link between the commonest risk allele and the effector, and with it much of the argument's claim on sporadic disease.

10.3 The repair prediction

The interventional form of the claim is the one that matters clinically.

The prediction: restoring endosomal recycling restores the *dynamic range* of the serine mark, not merely its mean. NHE6 inhibition has been shown to reverse the ApoE4-induced recycling block of ApoER2 and its associated glutamate receptors and to restore reelin-mediated modulation of excitatory synapses (Xian et al., 2018). The prediction here goes beyond that result: in ApoE4 neurons, NHE6 inhibition should restore the *excursion* of phospho-serine-3 in response to a plasticity-inducing stimulus, and should do so without a large change in baseline. If the excursion is not restored, then repairing the receptor limb is insufficient and the transducer limb must be addressed separately.

10.4 The timing prediction

The endosomal abnormality precedes deposition (Cataldo et al., 2000). The Ephexin5 arm is gated on amyloid, since its first step requires amyloid to deplete the licensing receptor. These two facts make an ordering claim.

The prediction: in a model carrying humanised precursor protein at endogenous levels, endosomal enlargement and impaired ApoER2 recycling precede the elevation of Ephexin5, which in turn precedes measurable dispersion of phospho-serine-3. If Ephexin5 elevation is found to precede any detectable sorting abnormality, the disposal-clock framing is wrong about order, and the elevation must be explained transcriptionally rather than proteolytically.

XI. The discriminating experiments

Seven experiments, ranked by what they settle per unit of effort. Each is stated so that a specific result would count against the argument.

1. **Paired-residue quantification in human cortex.** Array tomography of Alzheimer and age-matched control cortex, staining phospho-tyrosine-361 Ephexin5, total Ephexin5, phospho-serine-3 cofilin, total cofilin, a synaptic marker and LilrB2. Report the *joint distribution* of the two phospho-marks across single excitatory synapses, not the two means. In parallel, active-RhoA and active-Cdc42 pulldowns from the same tissue blocks. **Refutes if** the two marks covary in disease as in control. This is the primary falsifier and it requires one antibody the field does not yet have in validated form — against the licensing tyrosine — and nothing else that is not routine.

2. **The kinase-substitution test.** In amyloid-treated mature hippocampal neurons, inhibit Fyn and measure phospho-tyrosine-361 Ephexin5, total Ephexin5, active RhoA and active Cdc42. The hypothesis of Section 6.3 predicts that Fyn inhibition lowers the phosphorylated fraction without lowering total protein, and shifts the GTPase balance toward Cdc42. **Refutes the hypothesis if** phospho-tyrosine-361 is unchanged by Fyn inhibition, in which case another kinase supplies the RhoA bias and should be sought.

3. **Co-manipulation of the two arms in one preparation.** Manipulate ApoER2/Disabled-1 signalling and LilrB2 signalling in the same neurons and measure both governing tyrosines and the serine. The two pathways have never been run in the same preparation. **Refutes the convergence** if they do not interact, in which case the arms are independent and should be treated separately.

4. **The disposal-route test for the effector.** Apply a membrane-impermeant neuronal-membrane-proteasome inhibitor to mature hippocampal neurons and measure dendritic Ephexin5 with and without chemical long-term potentiation. If the activity-dependent fall in dendritic Ephexin5 is abolished by surface-restricted inhibition, the hypothesis of Section 7.3 is supported; if it survives surface-restricted inhibition but is abolished by broad proteasome inhibition, the canonical proteasome is responsible and that hypothesis is refuted.

5. **The genotype ordering.** Measure Ephexin5, total and phospho-tyrosine-361, in ApoE2, ApoE3 and ApoE4 knock-in neurons, unchallenged and after amyloid. **Refutes** the link between the commonest risk allele and this effector if no ordering is present.

6. **Repair before signal.** In ApoE4 neurons, compare three interventions on the excursion of phospho-serine-3 to a plasticity-inducing stimulus: NHE6 inhibition alone; exogenous reelin alone; and both. The account predicts that reelin alone gives little, that NHE6 inhibition alone restores excursion, and that the combination is superior to either. **Refutes** the ordering claim if exogenous reelin restores excursion in a cell whose receptor recycling is still blocked.

7. **Separate the two sugars.** Treat with chondroitinase, which removes the perineuronal net and leaves heparan sulfate, and separately with heparinase, which does the reverse; measure reelin-induced serine-3 phosphorylation after each. **Predicts** that reelin signalling survives chondroitinase and fails under heparinase — that is, that reelin does not require a perineuronal net at all. **Refutes the matrix arm** if chondroitinase abolishes the signal.

XII. What this forbids

A claim that forbids nothing is not worth grading. This one forbids five things, and three of them are being contemplated or done now.

It forbids lowering Ephexin5 as a therapeutic strategy. Heterozygous loss-of-function mutations in *ARHGEF15* cause autosomal-dominant cerebral small-vessel disease with osteoporotic fracture, through RhoA/ROCK2 inactivation (Ding et al., 2023). Chronic systemic reduction of this exchange factor is a phenocopy of a human dementing vasculopathy with skeletal fragility. Separately, the 2025 revision shows the protein is required for activity-driven spine growth, so the neuronal cost is an ablation of the growth arm rather than a removal of a brake (Petshow et al., 2025).

It forbids total-protein readouts of any transducer in this system. Where one covalent mark sets both what an enzyme does and how long it survives, abundance and activity are two readings of one modification and cannot be targeted or interpreted independently. Every published measurement of "Ephexin5 levels" in this disease is a measurement of total protein.

It forbids treating bulk phospho-cofilin as an endpoint. Section II establishes that the mark is the shared output of two antagonistic arms and of a protective arm, so its level is uninformative about direction even when measured perfectly. Any trial or biomarker programme reporting mean phospho-serine-3 cofilin from homogenate is reporting a quantity this account predicts is uninterpretable.

It forbids inducing autophagy or degradative flux before the compartment can complete it. If the failing step is terminal — sorting, acidification, hydrolysis — then increasing induction fills the neuron faster with vesicles it cannot empty. The order must be repair, then induce.

And it forbids the inference from a shared kinase to a shared target. Fyn serves the amyloid-driven destructive arm and the reelin-dependent protective arm, and it both applies and terminates the protective signal. Inhibiting it is predicted to help one stratum and harm another, which is what a failed trial with a real positive subgroup looks like.

XIII. Scope: what portion of the disease this claims

The largest failure mode of theories in this field is claiming everything and forbidding nothing. This section states the boundary.

What is claimed. This account applies to the *execution* of synaptic loss — the mechanical step by which an upstream cause becomes a lost spine. Synapse loss is the strongest structural correlate of cognitive impairment in this disease, with neocortical synapse density reaching a multivariate correlation of 0.96 against the Dementia Rating Scale and plaque density contributing 26 per cent of that model's strength (Terry et al., 1991). To the extent that dementia in Alzheimer's disease is caused by synapse loss, this account claims the final common step and the specific reason its central measurement has been unreadable.

What is not claimed, and these are large.

Initiation. Nothing here says what starts the disease. The account is compatible with amyloid-first, tau-first, vascular-first and inflammatory-first origins — a weakness as well as a strength, since a claim compatible with every upstream story constrains none of them. The one ordering commitment it does make is stated as a prediction in Section 10.4 and can be broken.

Selective vulnerability. It does not explain why particular neurons and circuits fail first. Cofilin is ubiquitous; the disease is not. The ApoER2 expression map is the most suggestive available answer and it is not this paper's (Ramsden et al., 2022); it also has a stated gap, since ApoER2-expressing neurons are spared even in the same layer of the same section, so high expression is necessary but plainly not sufficient.

The non-synaptic contribution. Neuronal death, white-matter injury and the loss of whole compartments contribute to dementia by routes this account does not describe.

Dementia not attributable to Alzheimer pathology. A substantial minority of dementia in community-based series is not explained by the pathologies conventionally measured, and a substantial minority of people meeting neuropathological criteria are not demented at death. This account speaks to neither group.

The brainstem interval. The tangle pathology of this disease begins in the brainstem decades before cortical amyloid is detectable. The Ephexin5 arm is gated on amyloid — its first step requires amyloid to deplete EphB2 — so it cannot begin before there is enough soluble oligomer in the

relevant compartment. Whatever begins the disease is operating in a compartment and at a time this account does not address. It executes; it does not initiate.

The honest summary of scope: this is a claim about the last step of one route, that route being the one most of the field's structural evidence points at, with no claim over who enters the route or why. A reader who wants a theory of the disease's cause will not find one here, and should not be told they have.

13.1 The inhibitory neuron, and why it is not forgotten

The most frequent objection to a spine-centred account is that the cortex's most conspicuously vulnerable inhibitory cell — the parvalbumin-positive fast-spiking interneuron — is aspiny, and that an argument about spine actin therefore misses it. The objection is correct as far as it goes and is accepted here rather than argued around.

That cell enters this account in one way only. The perineuronal net is a modifier of ligand exposure upstream of the residues discussed, not the site of the lesion. Its degradation removes the protection of the interneuron it sheathes; the resulting failure of inhibition raises the activity of the pyramidal population; and heightened pyramidal activity increases the local production of the very ligands — amyloid-β, and with age and disease the complement fragment C4d — that occupy LilrB2 on pyramidal dendrites (Brott et al., 2025). The matrix is upstream of the ligand, and the ligand is upstream of the residues.

A minority of excitatory neurons carry a net of their own, and in human frontal cortex those neurons carry conspicuously little phospho-tau (de Vries et al., 2024). They are the only population in which spines, a net and reelin-responsiveness are properties of a single cell, and they are therefore the natural internal control for any measurement proposed here. No claim is made that the parvalbumin interneuron fails by the mechanism described in this paper. It probably does not.

XIV. What would refute this

Written as a list of results that would end the argument, and stated so that each is achievable with existing methods.

1. **The two phospho-marks covary in Alzheimer cortex as they do in control.** The degeneracy of the serine then has no consequence in real tissue, the sign is recoverable where the field has been looking, and the paper's central proposal is unnecessary. This is the primary falsifier.

2. **Total Ephexin5 predicts active RhoA as well as the phospho-tyrosine fraction does.** The licensing architecture is then not load-bearing in disease, and the objection to total-protein measurement in Section XII falls with it.

3. **The reelin-induced destruction of Disabled-1 is shown not to occur in mature neurons.** The parallel between the two transducers is then developmental only, and the general claim that this control surface terminates by proteolysis loses one of its two pillars. The published demonstration is in cultured primary embryonic neurons (Bock et al., 2004); it has not, to our knowledge, been repeated in adult tissue, and this is the argument's most exposed empirical assumption.

4. **Restoring endosomal recycling fails to restore the excursion of phospho-serine-3.** Repair of the receptor limb is then insufficient, the therapeutic proposal of Section XVI is wrong in its emphasis, and the transducer limb must carry the argument alone.

5. **Ephexin5 elevation is shown to precede any sorting abnormality**, or to be transcriptional rather than proteolytic in origin. The misdirection claim of Section IX then has the order wrong.

6. **A capacity-deficit model is shown to predict the EphB2 and Ephexin5 directions jointly.** The specificity claim is then unnecessary and the simpler framing should be preferred.

Any one of 1, 2 or 3 removes the paper's central proposal. Items 4 and 6 remove its necessity; item 5 removes its ordering.

XV. Ledger of claims

Every load-bearing claim, with its evidence and grade. **M1** human population-scale or multi-cohort; **M2** human single cohort or tissue series; **M3** animal replicated; **M4** animal single laboratory; **M5** in vitro or inference.

claim	source	grade	note
Synapse loss is the strongest structural correlate of cognitive impairment	Terry et al., 1991	**M2**	$r = 0.96$ multivariate; plaques 26% of model strength
Cofilin is switched at serine 3; phosphorylation prevents F-actin binding	Chai et al., 2009; Bamburg et al., 2021	**M5**	Established biochemistry
RhoA→ROCK→LIMK and Rac1/Cdc42→PAK→LIMK both raise pSer3 with opposite structural outcomes	Kang and Woo, 2019; Bamburg et al., 2021	**M5**	Canonical Rho-family signalling; the degeneracy of §II
Aβ→LilrB2 enhances cofilin signalling (dephosphorylation), seen in human AD brain	Kim et al., 2013	**M2**	Human observation is of cofilin signalling, not a per-synapse ratio
The dephosphorylation direction reproduces in an independent laboratory	Kawaguchi et al., 2022	**M4**	Antagonist rather than knockout
Aβ→ROCK increases pSer3 in human AD cortex; necessary and sufficient for impairment	Rush et al., 2018	**M2**	Post-synaptic-enriched synaptosome fraction; opposite direction to the row above
C4d binds LilrB2/PirB, is elevated in AD, and is sufficient to strip spines	Brott et al., 2025	**M2 / M3**	Human colocalisation M2; sufficiency in mouse M3, abolished in PirB-null
Cofilin–actin rods require active (dephosphorylated) cofilin and are present in human AD brain	Minamide et al., 2000; Bamburg and Bernstein, 2016	**M2 / M4**	Most prominent in neurites contacting amyloid
Aβ signalling engages the phosphatase limb via SSH1 and RanBP9	Woo et al., 2015; Kang and Woo, 2019	**M3 / review**	RanBP9 reduction protects in an amyloid model

claim	source	grade	note
PP2A is the principal brain tau phosphatase and shows multiple abnormalities in AD	Torrent and Ferrer, 2012	**Review**	The reviewers note the individual alterations are not uniformly replicated
EphB signalling phosphorylates Ephexin5, recruiting Ube3A for proteasomal destruction	Margolis et al., 2010	**M4**	Biochemistry with ligase identification
Ephexin5 activates RhoA and Cdc42; substrate choice is set by tyrosine phosphorylation; Y361F biases to Cdc42	Petshow et al., 2025	**M4**	FRET biosensors at single spines; Ephexin5 is *required* for activity-driven spine growth
The residue that selects the substrate is the residue that licenses destruction	Margolis et al., 2010; Petshow et al., 2025	**Observation**	Verifiable from the two sources; abundance and activity are one variable
Dendritic Ephexin5 falls after activity in a proteasome-dependent manner	Hamilton et al., 2017	**M4**	The controlled variable is dendritic; the control is proteolytic
Ephexin5 protein is elevated in human Alzheimer hippocampus	Sell et al., 2017	**M2**	Single report; no independent cohort; no cell-type resolution
Aβ binds EphB2's fibronectin repeats and triggers its proteasomal degradation; restoring EphB2 rescues	Cissé et al., 2011	**M3**	Bidirectional manipulation in vivo
***ARHGEF15* heterozygous loss of function causes dominant cerebral small-vessel disease with osteoporosis, via RhoA/ROCK2 inactivation**	Ding et al., 2023	**M1**	Two families plus two sporadic cases; transgenic mouse recapitulates
Reelin binds ApoER2/VLDLR, induces Dab1 tyrosine phosphorylation, and its loss hyperphosphorylates tau	Hiesberger et al., 1999	**M4**	The GSK-3β limb of the protective arm
Reelin/ApoER2/Dab1 phosphorylates cofilin serine 3 and stabilises actin	Chai et al., 2009	**M4**	Developmental context; not shown in adult disease
Reelin targets Dab1 for ubiquitin–proteasome degradation; Tyr phosphorylation is required; Fyn deficiency prevents it	Bock et al., 2004	**M5/M4**	Primary embryonic neurons; proteasome blockade prevents cortical-plate formation

claim	source	grade	note
Fyn is the physiological Dab1 kinase; Dab1 protein rises in Fyn-deficient mice	Arnaud et al., 2003; Bock and Herz, 2003	**M4**	Src partially redundant when Fyn copy number falls
Both transducers above Ser3 are terminated by phosphorylation-licensed proteolysis, and Fyn writes to both	This paper	**Observation**	Verifiable from the four sources above; §V.2
Aβ–PrP^{C}–mGluR5 activates Fyn at the PSD and drives spine loss	Um et al., 2013	**M3**	mGluR5 antagonism reverses deficits in transgenic mice
Tau targets Fyn to the post-synaptic density; removing tau uncouples Aβ toxicity	Ittner et al., 2010	**M3**	Rescued by a PSD-95/NR uncoupling peptide
Enlarged early endosomes are the earliest disease-specific change; before deposition; from 28 weeks in Down syndrome; absent in advanced PSEN familial AD	Cataldo et al., 2000	**M2**	The presenilin control establishes independence from Aβ deposition; APOE ε4 accentuates
***SORL1* loss of function in human neurons enlarges endosomes; full loss adds lysosomal and autophagic failure; relieved by lowering APP**	Hung et al., 2021	**M2**	Isogenic human neurons; places PSEN1, APP and SORL1 in one pathway
SORLA loss impairs lysosomal degradation and exocytosis in human microglia; synaptosomes accumulate undegraded	Mishra et al., 2025	**M2**	The clearing cell acquires the same lesion as the losing cell
ApoE4 sequesters ApoER2 intracellularly, reducing surface receptor and reelin's enhancement of glutamate signalling	Chen et al., 2010	**M3**	A trafficking lesion, not a binding lesion
NHE6 inhibition reverses the ApoE4 recycling block and restores reelin's modulation of excitatory synapses	Xian et al., 2018	**M3**	The only cited intervention that restores capacity rather than adding signal
Reactive lipid aldehydes crosslink ApoE to ApoER2; pathway components accumulate in perforant-path terminal zones and correlate with progression	Ramsden et al., 2022	**M5 / M2**	Chemistry M5 (purified peptides, driven concentrations); human IHC M2; no crosslink isolated from brain

claim	source	grade	note
Aβ42 accumulates in multivesicular bodies of post-synaptic compartments before plaques	Takahashi et al., 2002	**M2**	Immunoelectron microscopy in human, mouse and rat
TFEB loss reduces lysosomal exocytosis of tau and worsens intraneuronal pathology and spreading	Xu et al., 2021	**M3**	Establishes the route as clearance, not release
A tauopathy-associated tau fragment blocks TFEB nuclear translocation and lysosomal capacity	Pollack et al., 2024	**M5**	Cell lines and primary neurons; the feed-forward loop
The neuronal membrane proteasome is dendritic, activity-regulated, and its peptides activate NMDA receptors	Türker et al., 2024	**M5**	Candidate for the rapid activity-dependent Ephexin5 route — **not shown**
N-sulfated heparan sulfate is an obligate co-receptor for reelin-induced ApoER2 dimerisation	Pan et al., 2025	**M5**	K_D 17 nM, COLBOS 10 nM; heparinase, NDST1-KO and free heparin block; N-desulfated heparin does not
The perineuronal net is condensed chondroitin sulfate; HSPGs are diffuse-ECM components	Fawcett et al., 2022	**Review**	Basis for the claim that reelin does not require a net
Excitatory neurons bearing a perineuronal net carry low phospho-tau in human frontal cortex	de Vries et al., 2024	**M2**	The internal control for the proposed measurements
A RELN gain-of-function allele confers resistance to autosomal-dominant AD	Lopera et al., 2023	**M2**	n = 1 index case; stronger Dab1 activation
ROCK1/2 are elevated in AD and the inhibitor class is in development	Zheng et al., 2025	**Review**	The review itself states "activation or inhibition" alters synaptic structure
Alzheimer's synaptic execution is a lesion of proteolytic *specificity*, not of proteolytic capacity	This paper	**Inference (M5)**	The paper's original claim. Untested. §XIV states its falsifiers

claim	source	grade	note
The phospho-Y361 / phospho-Ser3 covariance falls in AD while Ser3 dispersion rises	This paper	**Prediction**	Never measured; requires one new validated antibody
Fyn phosphorylates Y361 without licensing Ube3A recruitment in the amyloid-bearing dendrite	This paper	**Hypothesis**	The candidate for the missing RhoA bias of §4.4; §XI.2 tests it
Dendritic Ephexin5 is ordered by APOE genotype before any amyloid challenge	This paper	**Prediction**	Knock-in neurons already exist

Four entries in this table are the paper's own, and none has been tested. That is stated here rather than in a discussion section, and they are the first things a reader should attack.

XVI. What follows for treatment

16.1 The category error to avoid

The reflex at a dysregulated node is to block it. At this control surface the reflex fails three times over, and it is worth being explicit about why, because each failure has a live programme attached to it.

Blocking the effector fails because the effector is non-monotonic. Cofilin severs filaments at low occupancy and stabilises them at high occupancy (Bamburg and Bernstein, 2016). A drug that pushes every synapse one way along a curve that turns over must convert some fraction of synapses from one failure mode into the other.

Blocking the exchange factor fails because the human loss-of-function phenotype is a dementing vasculopathy (Ding et al., 2023), and because the protein is required for the spine growth that accompanies potentiation (Petshow et al., 2025).

Blocking the kinase fails because the kinase is shared. Fyn transduces amyloid toxicity at the post-synaptic density and is simultaneously required for the reelin arm to be applied and terminated (Ittner et al., 2010; Um et al., 2013; Arnaud et al., 2003; Bock et al., 2004).

There is a pattern in these three failures. Every one of them is an attempt to fix a *timing* problem with a *level* intervention. The system's defect, on the account given here, is that instructions are not being ended. Lowering the amplitude of an instruction that will not end is not the same as ending it.

16.2 The target implied: repair the clock, then supply the signal

The intervention category this account points to is the restoration of the cell's capacity to terminate signals — and one published result already occupies it.

Inhibition of NHE6, the early endosome's principal proton-leak channel, completely reverses the ApoE4-induced recycling block of ApoER2 and of the glutamate receptors co-endocytosed with it, and restores reelin's modulation of excitatory synapses (Xian et al., 2018). Nothing was added to the system and nothing was blocked in the signalling pathway. The compartment was repaired and the signalling returned.

That result deserves more weight than it has received, and this account explains why it should work: an ApoE4 neuron does not have a reelin deficiency, it has a receptor in the wrong place, and no quantity of ligand fixes a receptor in the wrong place.

The ordering claim follows directly and is the most immediately actionable statement in this paper. **Repair capacity first; supply signal second.** A reelin-directed agonist, a pathway-activating antibody or any other attempt to drive the protective arm should be expected to underperform in exactly the population most likely to be enrolled — ApoE4 carriers — unless the recycling defect is addressed first or concurrently. The same ordering applies to the degradative arm: an autophagy inducer given to a neuron that cannot complete the pathway fills it faster with vesicles it cannot empty.

16.3 The genetic anchor, and what it does and does not license

The human genetic anchor for the protective arm is of unusual quality. A gain-of-function *RELN* variant was carried by a man who resisted an autosomal-dominant Alzheimer mutation into his late sixties despite very high amyloid burden, with stronger Disabled-1 activation and reduced tau phosphorylation in a knock-in mouse (Lopera et al., 2023).

On the reading developed here, that allele should be expected to act by improving the *efficiency of a transient signal* rather than by raising tone — because the pathway it enters is one in which the transducer is consumed by the act of signalling. This yields a specific prediction that distinguishes the two readings: a knock-in animal carrying the variant should show a larger reelin-induced *excursion* at cofilin serine 3 with a faster return to baseline and, on the account of Section V.2, faster Disabled-1 turnover — not a tonic elevation of phospho-cofilin. If instead the animal shows tonically raised phospho-cofilin with no improvement in dynamic range, the reading offered here is wrong and the allele is doing something simpler.

16.4 The drug conflict that has not been named

Reelin's delivery route creates a conflict with a strategy currently attractive for tau.

Reelin requires N-sulfated heparan sulfate as an obligate co-receptor for ApoER2 dimerisation, and heparinase or free heparin reduces reelin-induced dimerisation while N-desulfated heparin does not (Pan et al., 2025). Heparan-sulfate proteoglycans are also the route by which pathological tau enters neurons, which has made heparin mimetics attractive for blocking transcellular tau propagation.

A heparin mimetic given to stop tau spreading would, by the identical chemistry, silence the reelin arm that restrains tau phosphorylation through Disabled-1 and glycogen synthase kinase-3β. That is a specific, verified, drug-level contradiction, and it argues that the sulfated matrix must be modulated rather than blocked. It also disposes of a common conflation: the perineuronal net is chondroitin sulfate, reelin's requirement is heparan sulfate, and reelin therefore does not need a net in order to signal (Fawcett et al., 2022).

16.5 And the endpoints must change before the drugs do

Three measurement practices should be retired in programmes at this node, and each follows from a specific argument above rather than from general caution.

Total protein for a transducer whose abundance and activity are one variable. Where the mark that activates also condemns, total Ephexin5 and total Disabled-1 are uninterpretable, and an elevation is as consistent with a failed signal as with an amplified one.

Mean phospho-serine-3 cofilin from homogenate. The mark is the shared output of two antagonistic arms and a protective one; its mean is uninformative about direction even when measured perfectly.

Bulk degradative-capacity assays. Total proteasome activity, total autophagic flux and total lysosomal enzyme content cannot detect a change in the *specificity* of proteolysis, which is what Section IX argues the disease produces.

What should replace them is a joint, per-synapse measurement of the governing tyrosine and the governed serine, with the GTPase state determined in the same tissue. That is more difficult than what is done now. It is not, on the evidence assembled here, optional: until it is measured, the field is choosing the direction of a therapy from a variable that cannot carry the direction.

References

Arnaud L, Ballif BA, Förster E, Cooper JA. Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Curr Biol*. 2003;13(1):9–17. PMID 12526739. doi:10.1016/s0960-9822(02)01397-0

Bamburg JR, Bernstein BW. Actin dynamics and cofilin-actin rods in Alzheimer disease. *Cytoskeleton (Hoboken)*. 2016;73(9):477–497. PMID 26873625. doi:10.1002/cm.21282

Bamburg JR, Minamide LS, Wiggan O, Tahtamouni LH, Kuhn TB. Cofilin and actin dynamics: multiple modes of regulation and their impacts in neuronal development and degeneration. *Cells*. 2021;10(10):2726. PMID 34685706. doi:10.3390/cells10102726

Bock HH, Herz J. Reelin activates SRC family tyrosine kinases in neurons. *Curr Biol*. 2003;13(1):18–26. PMID 12526740. doi:10.1016/s0960-9822(02)01403-3

Bock HH, Jossin Y, May P, Bergner O, Herz J. Apolipoprotein E receptors are required for reelin-induced proteasomal degradation of the neuronal adaptor protein Disabled-1. *J Biol Chem*. 2004;279(32):33471–33479. PMID 15175346. doi:10.1074/jbc.M401770200

Brott BK, Raissi AJ, Micheva KD, Vielmetter J, Mendes MS, Baccus CJ, Huang J, Shatz CJ. C4d, a high-affinity LilrB2 ligand, is elevated in Alzheimer's disease and mediates synapse pruning. *Proc Natl Acad Sci U S A*. 2025;122(38):e2519253122. PMID 40966293. doi:10.1073/pnas.2519253122

Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. Endocytic pathway abnormalities precede amyloid β deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *Am J Pathol*. 2000;157(1):277–286. PMID 10880397. doi:10.1016/s0002-9440(10)64538-5

Chai X, Förster E, Zhao S, Bock HH, Frotscher M. Reelin stabilizes the actin cytoskeleton of neuronal processes by inducing n-cofilin phosphorylation at serine3. *J Neurosci*. 2009;29(1):288–299. PMID 19129405. doi:10.1523/JNEUROSCI.2934-08.2009

Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A*. 2010;107(26):12011–12016. PMID 20547867. doi:10.1073/pnas.0914984107

Cissé M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu GQ, Mucke L. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011;469(7328):47–52. PMID 21113149. doi:10.1038/nature09635

de Vries LE, Bahnerth A, Swaab DF, Verhaagen J, Carulli D. Resilience to Alzheimer's disease associates with alterations in perineuronal nets. *Alzheimers Dement*. 2024;21(2):e14504. PMID 39737731. doi:10.1002/alz.14504

Ding X, Chen Y, Guo C, Fu Y, Qin C, Zhu Q, Wang J, Zhang R, Tian H, Feng R, Liu H, Liang D, Wang G, Teng J, Li J, Tang B, Wang X. Mutations in ARHGEF15 cause autosomal dominant hereditary cerebral small vessel disease and osteoporotic fracture. *Acta Neuropathol*. 2023;145(5):681–705. PMID 36929019. doi:10.1007/s00401-023-02560-6

Fawcett JW, Fyhn M, Jendelova P, Kwok JCF, Ruzicka J, Sorg BA. The extracellular matrix and perineuronal nets in memory. *Mol Psychiatry*. 2022;27(8):3192–3203. PMID 35760878. doi:10.1038/s41380-022-01634-3

Hamilton AM, Lambert JT, Parajuli LK, Vivas O, Park DK, Stein IS, Jahncke JN, Greenberg ME, Margolis SS, Zito K. A dual role for the RhoGEF Ephexin5 in regulation of dendritic spine outgrowth. *Mol Cell Neurosci*. 2017;80:66–74. PMID 28185854. doi:10.1016/j.mcn.2017.02.001

Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron*. 1999;24(2):481–489. PMID 10571241. doi:10.1016/s0896-6273(00)80861-2

Hung C, Tuck E, Stubbs V, van der Lee SJ, Aalfs C, van Spaendonk R, Scheltens P, Hardy J, Holstege H, Livesey FJ. SORL1 deficiency in human excitatory neurons causes APP-dependent defects in the endolysosome-autophagy network. *Cell Rep*. 2021;35(11):109259. PMID 34133918. doi:10.1016/j.celrep.2021.109259

Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wölfing H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Götz J. Dendritic function of tau mediates amyloid-β toxicity in Alzheimer's disease mouse models. *Cell*. 2010;142(3):387–397. PMID 20655099. doi:10.1016/j.cell.2010.06.036

Kang DE, Woo JA. Cofilin, a master node regulating cytoskeletal pathogenesis in Alzheimer's disease. *J Alzheimers Dis*. 2019;72(s1):S131–S144. PMID 31594228. doi:10.3233/JAD-190585

Kawaguchi Y, Matsubayashi J, Kawakami Y, Nishida R, Kurihara Y, Takei K. LOTUS suppresses amyloid β-induced dendritic spine elimination through the blockade of amyloid β binding to PirB. *Mol Med*. 2022;28(1):154. PMID 36510132. doi:10.1186/s10020-022-00581-7

Kim T, Vidal GS, Djurisic M, William CM, Birnbaum ME, Garcia KC, Hyman BT, Shatz CJ. Human LilrB2 is a β-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. *Science*. 2013;341(6152):1399–1404. PMID 24052308. doi:10.1126/science.1242077

Lopera F, Marino C, Chandrahas AS, O'Hare M, Villalba-Moreno ND, Aguillon D, et al. Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man. *Nat Med*. 2023;29(5):1243–1252. PMID 37188781. doi:10.1038/s41591-023-02318-3

Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR, Hu L, Greer PL, Bikoff JB, Ho HH, Soskis MJ, Sahin M, Greenberg ME. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell*. 2010;143(3):442–455. PMID 21029865. doi:10.1016/j.cell.2010.09.038

Minamide LS, Striegl AM, Boyle JA, Meberg PJ, Bamburg JR. Neurodegenerative stimuli induce persistent ADF/cofilin-actin rods that disrupt distal neurite function. *Nat Cell Biol*. 2000;2(9):628–636. PMID 10980704. doi:10.1038/35023579

Mishra S, Morshed N, Sidhu SB, Kinoshita C, Stevens B, Jayadev S, Young JE. The Alzheimer's disease gene SORL1 regulates lysosome function in human microglia. *Glia*. 2025;73(7):1329–1348. PMID 40183375. doi:10.1002/glia.70009

Pan L, Song X, Su G, Gandy LA, Fang B, Buttaci M, Gibson J, Xia K, Zhang F, Liu J, Wang L, Temple S, Wang C. N-Sulfated heparan sulfate promotes Reelin signaling as a co-receptor. *J Am Chem Soc*. 2025;147(51):46773–46779. PMID 41359976. doi:10.1021/jacs.5c15573

Pesold C, Liu WS, Guidotti A, Costa E, Caruncho HJ. Cortical bitufted, horizontal, and Martinotti cells preferentially express and secrete reelin into perineuronal nets, nonsynaptically modulating gene expression. *Proc Natl Acad Sci U S A*. 1999;96(6):3217–3222. PMID 10077664. doi:10.1073/pnas.96.6.3217

Petshow S, Coblentz A, Hamilton AM, Sarkar D, Anisimova M, Flores JC, Zito K. Activity-dependent regulation of Cdc42 by Ephexin5 drives synapse growth and stabilization. *Sci Adv*. 2025;11(13):eadp5782. PMID 40138406.

doi:10.1126/sciadv.adp5782

Pollack SJ, Dakkak D, Guo T, Chennell G, Gomez-Suaga P, Noble W, Jimenez-Sanchez M, Hanger DP. Truncated tau interferes with the autophagy and endolysosomal pathway and results in lipid accumulation. *Cell Mol Life Sci*. 2024;81(1):304. PMID 39009859. doi:10.1007/s00018-024-05337-6

Ramsden CE, Keyes GS, Calzada E, Horowitz MS, Zamora D, Jahanipour J, Sedlock A, Indig FE, Moaddel R, Kapogiannis D, Maric D. Lipid peroxidation induced ApoE receptor-ligand disruption as a unifying hypothesis underlying sporadic Alzheimer's disease in humans. *J Alzheimers Dis*. 2022;87(3):1251–1290. PMID 35466940. doi:10.3233/JAD-220071

Rush T, Martinez-Hernandez J, Dollmeyer M, Frandemiche ML, Borel E, Boisseau S, Jacquier-Sarlin M, Buisson A. Synaptotoxicity in Alzheimer's disease involved a dysregulation of actin cytoskeleton dynamics through cofilin 1 phosphorylation. *J Neurosci*. 2018;38(48):10349–10361. PMID 30341179. doi:10.1523/JNEUROSCI.1409-18.2018

Sell GL, Schaffer TB, Margolis SS. Reducing expression of synapse-restricting protein Ephexin5 ameliorates Alzheimer's-like impairment in mice. *J Clin Invest*. 2017;127(5):1646–1650. PMID 28346227. doi:10.1172/JCI85504

Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer Aβ42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol*. 2002;161(5):1869–1879. PMID 12414533. doi:10.1016/s0002-9440(10)64463-x

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991;30(4):572–580. PMID 1789684. doi:10.1002/ana.410300410

Torrent L, Ferrer I. PP2A and Alzheimer disease. *Curr Alzheimer Res*. 2012;9(2):248–256. PMID 22299660. doi:10.2174/156720512799361682

Türker F, Brennan A, Margolis SS. Neuronal membrane proteasome-derived peptides modulate NMDAR-dependent neuronal signaling to promote changes in gene expression. *Mol Biol Cell*. 2024;35(1):ar6. Epub 2023 Nov 1. PMID 37910253. doi:10.1091/mbc.E23-06-0218

Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, Kerrisk ME, Vortmeyer A, Wisniewski T, Koleske AJ, Gunther EC, Nygaard HB, Strittmatter SM. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer Aβ oligomer bound to cellular prion protein. *Neuron*. 2013;79(5):887–902. PMID 24012003. doi:10.1016/j.neuron.2013.06.036

Woo JA, Boggess T, Uhlar C, Wang X, Khan H, Cappos G, Joly-Amado A, De Narvaez E, Majid S, Minamide LS, Bamburg JR, Morgan D, Weeber E, Kang DE. RanBP9 at the intersection between cofilin and Aβ pathologies: rescue of neurodegenerative changes by RanBP9 reduction. *Cell Death Dis*. 2015;6(3):e1676. PMID 25741591. doi:10.1038/cddis.2015.37

Xian X, Pohlkamp T, Durakoglugil MS, Wong CH, Beck JK, Lane-Donovan C, Plattner F, Herz J. Reversal of ApoE4-induced recycling block as a novel prevention approach for Alzheimer's disease. *eLife*. 2018;7:e40048. PMID 30375977. doi:10.7554/eLife.40048

Xu Y, Du S, Marsh JA, Horie K, Sato C, Ballabio A, Karch CM, Holtzman DM, Zheng H. TFEB regulates lysosomal exocytosis of tau and its loss of function exacerbates tau pathology and spreading. *Mol Psychiatry*. 2021;26(10):5925–5939. Epub 2020 May 4. PMID 32366951. doi:10.1038/s41380-020-0738-0

Zheng C, Xia W, Zhang J. Rock inhibitors in Alzheimer's disease. *Front Aging*. 2025;6:1547883. PMID 40182055. doi:10.3389/fragi.2025.1547883

Every reference above was checked against its PubMed record — title, authorship, year, journal, volume, pages and the direction of the reported effect — rather than recalled. The two disagreements this paper turns on, at cofilin serine 3 and over the sign of the Ephexin5 model, are the reason that check was necessary: in both cases the published directions are opposite, and a citation reconstructed from memory would have silently resolved a dispute that the literature has not resolved.