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The Address and the Switch

*Cortical Immature Neurons, Disease-Associated Microglia, and the
Reelin–Heparan-Sulfate–Ephexin5 Axis of Layer II — An Audit of a Five-Joint
Mechanism in Which Four of the Joints Run Backwards*

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Layer II is not a cell type but an address. The switch that governs it runs the other way.

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Abstract

A mechanism has been assembled, in reviews and in the informal synthesis that circulates ahead of them, which joins five of the most active areas in current Alzheimer research into a single causal chain. The chain runs: cortical immature neurons of layer II constitute a structural reserve; disease-associated microglia degrade the extracellular matrix on which that reserve depends; the degradation removes the sulfated sugar that reelin requires to signal; without reelin, the guanine-nucleotide exchange factor Ephexin5 is locked into activating RhoA; RhoA collapses the actin cytoskeleton through cofilin, the reserve cannot wake, and the patient becomes amnesic. Exercise, in the same chain, reverses every step. The chain is attractive because each of its joints is drawn from a real and well-conducted study, and because it terminates in an intervention that is free.

This paper audits the chain joint by joint against the primary literature. It finds that the terminal claim is defensible, that one joint is secure, and that four are inverted relative to the experiments that are cited in their support.

The first inversion is anatomical. *Layer II* is not a cell type but an address, and at least three distinct neuronal populations share it: the prenatally generated, doublecortin-positive cortical immature neurons; the reelin-expressing projection neurons of the anterolateral entorhinal cortex, which are the first cortical cells to tangle; and ordinary mature granular and pyramidal neurons. The chain's premises are drawn from all three and attributed to the first. A species constraint compounds the error: cortical immature neurons are essentially absent from the rodent neocortex and increase in linear density with brain size, so the rodent experiments that supply the chain's causal steps are performed in animals that do not possess the population the chain is about.

The second inversion is the receptor stalk. The O-linked sugar domain of ApoER2 is presented as a protective spacer whose proteolytic removal silences reelin. In the experiment that defines its function, deletion of that domain *reduced* extracellular cleavage of the receptor and *raised* receptor abundance in brain, with increased spine density and glutamate-receptor content. The domain promotes shedding; it does not shield against it.

The third and principal inversion is the switch itself. Ephexin5 does select between RhoA and Cdc42, and the selection is set by phosphorylation of a single tyrosine. But the direction is the opposite of the one asserted: it is the *non-phosphorylatable* mutant that enhances Cdc42 activation, and plasticity-inducing activity *reduces* tyrosine phosphorylation by roughly three-quarters within minutes. The same residue licenses the protein's own ubiquitin-dependent destruction, so abundance and substrate choice are two readings of one modification and cannot be targeted separately. The chain's invocation of the striatal-enriched phosphatase makes the inversion self-contradictory: a phosphatase that silences the tyrosine kinases should, on the published rule, bias the exchange factor *toward* Cdc42 and growth, not away from it.

The fourth inversion is the intervention. In the single-nucleus study that the chain treats as its empirical capstone, exercise *enhanced* the disease-associated microglial gene-expression signature rather than suppressing it, and the immature neurons whose profiles were restored were adult-born

neuroblasts of the dentate gyrus — a different population, in a different structure, in a species without neocortical immature neurons.

Against these, one joint is strengthened by the audit rather than weakened. Reelin and the microglial sensor TREM2 bind heparan sulfate through *non-overlapping* sulfation marks: reelin requires N-sulfation, for which 6-O-sulfation is not needed, while TREM2 requires 6-O-sulfation, for which N-sulfation is dispensable. The two are not competitors for one substrate. The competitor for the microglial mark is tau, whose internalisation requires the same 6-O-sulfation. The real contest on the polymer is between the sensor and the seed, and the protective ligand reads a different letter entirely.

We set out what the corrected chain supports, grade every load-bearing claim in an explicit validity ledger, and name for each inverted joint the measurement that would settle it. The most informative of these requires one antibody that does not yet exist. We argue that the chain's errors are not random: all four run in the direction that makes the mechanism tidier and the intervention more heroic, and that this is a property of how mechanisms are assembled across literatures that do not read one another.

I. The Chain as It Is Usually Drawn

There is a characteristic shape to the mechanisms that get assembled at the frontier of a crowded field. A finding is made in one laboratory and is secure. A second finding is made in another laboratory, in another preparation, and is also secure. Between them lies a gap that neither laboratory has crossed, and into that gap a connecting sentence is written — not dishonestly, and usually not even deliberately, but because the sentence is what makes the two findings into a story. Enough such sentences, and a chain exists which no single experiment supports and which no single reviewer is positioned to check, because checking it requires reading five literatures in their original rather than in each other's introductions.

The chain this paper audits has that shape. Stated at its most compact, it runs as follows.

Cortical layer II contains a population of neurons that were generated before birth and have never completed maturation. They retain the molecular markers of immaturity into adult life and can, on demand, finish differentiating and integrate into existing circuits. They are therefore a reserve — a bank of young cells held against future injury. In Alzheimer's disease, microglia transition from a homeostatic surveillance state into a disease-associated state, flooding the extracellular space with proteases and inflammatory mediators. These degrade the sulfated matrix and cleave the receptors on which reelin, the ligand that governs the reserve, depends. Reelin signalling fails. Downstream of that failure, the exchange factor Ephexin5 accumulates and is locked into activating RhoA rather than Cdc42; RhoA drives the severing of actin filaments; the immature cell cannot build a dendritic spine; the reserve cannot be drawn upon; and at the point where the reserve would have compensated, the patient converts from forgetful to amnesic. Physical exercise

reverses the microglial state, restores the matrix, restores reelin, flips the switch to Cdc42, and lets the reserve wake.

Every clause in that paragraph corresponds to a real paper. The cells exist and have been characterised across twelve mammalian species. The microglial state transition exists and was defined by single-cell transcriptomics in 2017. Reelin's dependence on a sulfated co-receptor was established in 2025. Ephexin5's substrate switch was established in the same year. The exercise study was published in *Nature Neuroscience* in 2025. There is no fabrication anywhere in the chain. What there is, instead, is a set of connecting sentences, and this paper is an audit of those sentences.

The audit's method is unglamorous: take each joint, find the experiment that is cited for it, and read what the experiment measured and in which direction the measurement went. Where the joint survives, say so. Where it does not, say which way the evidence actually runs and what would have to be true for the joint to be repaired. We have done this for five joints. One survives intact, one survives in a modified and more interesting form, and four are inverted — meaning not that the underlying finding is wrong but that the chain states its direction backwards.

We should say at the outset why this is worth a paper rather than a correction note. It is worth a paper because the chain is not idle. It names three therapeutic targets — the sulfotransferases, the exchange factor, and the phosphatase — and for each of the three, the inverted version of the mechanism prescribes the opposite intervention to the one the evidence supports. A programme that lowers Ephexin5 because the chain says Ephexin5 destroys synapses would be removing a protein that is required for activity-driven spine growth, and would be phenocopying a human vasculopathy while doing it. That is not a bookkeeping error.

It is also worth a paper because the errors are not randomly distributed. We return to this in the coda. All four inversions run in the same direction: toward a cleaner mechanism with a sharper villain and a more complete rescue. That is a signature, and it tells us something about how synthesis fails that a list of corrections would not.

II. The Address Three Populations That Share Layer II

The chain's first premise locates everything in cortical layer II. This is not an accident of exposition; layer II genuinely is where the disease's earliest cortical lesion appears, and it genuinely is where the immature neurons live. The problem is that these are not the same cells, and a third population — the ordinary mature neurons of the layer — supplies much of the pathology that the chain attributes to the other two.

The first population: cortical immature neurons. These are prenatally generated, non-dividing neurons that occupy layer II and persist in an arrested state of maturation into adult

life, expressing doublecortin and the polysialylated form of the neural cell adhesion molecule. They are not the product of adult neurogenesis; no stem cell makes them and none replaces them. They were systematically characterised across twelve mammalian species spanning lissencephalic to gyrencephalic brains (La Rosa and colleagues, 2020), and the finding that matters most for the present argument is their distribution: virtually absent from the rodent neocortex, present throughout the neocortex of many other species, with linear density in layer II increasing with brain size. In small-brained rodents they are restricted to the paleocortex — principally the piriform cortex, where they decline sharply through juvenile stages but persist in reduced numbers into advanced age (Ghibaudi and colleagues, 2023).

The second population: the reelin-expressing projection neurons of the anterolateral entorhinal cortex. These are mature excitatory projection neurons, and they are the predominant cortical site of tau hyperphosphorylation and tangle formation in prodromal Alzheimer's disease. A majority of layer II projection neurons in the anterolateral entorhinal cortex are unusual among cortical excitatory neurons in expressing reelin itself (Kobro-Flatmoen and colleagues, 2023). They are not immature, do not express doublecortin, and are not a reserve. They are the first cortical casualties.

The third population: ordinary granular and pyramidal neurons of layer II across the remaining cortical areas, which carry no special relationship to either reelin or immaturity.

The chain merges the first two. It takes the vulnerability of the second — the earliest tangles, the earliest intracellular amyloid, the dependence on reelin — and attributes it to the first, which supplies the reserve narrative. The merge is facilitated by the fact that both populations are correctly described as "layer II neurons expressing or depending on reelin," a sentence that is true of each in a different sense. The entorhinal cells *express* reelin as a secreted product; the immature cells are proposed to *depend* on reelin as a received signal. Those are different relationships to the same molecule, and conflating them produces the chain's central image — a dormant reserve held in its dormancy by a reelin brake — for which, as Section VII sets out, there is no direct evidence in either population.

The species constraint sharpens this considerably. Almost every causal experiment in the chain — the microglial state transition, the amyloid-driven loss of EphB2, the Ephexin5 manipulations, the exercise intervention — was performed in mice. Mice have essentially no neocortical cortical immature neurons (La Rosa and colleagues, 2020). Whatever those experiments demonstrate, they cannot demonstrate anything about a population the experimental animal does not possess in the region under study. This is not a pedantic objection: the trade-off hypothesis advanced for the comparative data holds that stem-cell-driven adult neurogenesis and the non-newly-generated immature reservoir vary inversely across mammals, as two alternative ways of keeping young neurons available (Bonfanti and colleagues, 2023). If that is right, the mouse is not a

scaled-down human for this purpose. It is an animal that solved the same problem differently, and the solution the chain is about is the one the mouse does not use.

The human population with the best documentation is not cortical at all. It is the paralaminar nucleus of the amygdala, where immature excitatory neurons, doublecortin-positive and polysialylated in childhood, transition into mature TBR1- and VGLUT2-positive neurons across adolescence while local progenitor proliferation falls sharply in infancy, and where immature cells persist into old age (Sorrells and colleagues, 2019). A homologous region has since been identified in the mouse and shown to be embryonically generated rather than postnatally produced, with a subset retaining immature features into adulthood and a subset migrating into the adjacent endopiriform cortex during juvenile and adolescent ages (Alderman and colleagues, 2023). This is the cleanest demonstration that the phenomenon is real, conserved, and protracted. It is also, pointedly, not in layer II of the neocortex, and not in any structure the chain discusses.

III. The Reserve What Is Known, and What Has Never Been Measured

Granting the population, what is actually known about its behaviour that would license calling it a reserve, and what is known about it in Alzheimer's disease?

The reserve interpretation rests on three observations. The cells retain markers of immaturity for years to decades without dividing. They can complete maturation and integrate into pre-existing circuits long after the developmental period has closed. And their abundance scales with brain size in the direction opposite to stem-cell neurogenesis, which is the pattern one would expect if they were an alternative solution to the same evolutionary problem (La Rosa and colleagues, 2020; Bonfanti and colleagues, 2023). Taken together these justify the phrase "reservoir of developmentally plastic cells," which is the phrase the primary literature actually uses, and which is weaker than "brain reserve" in a specific and important way: a reservoir is a quantity of material, while reserve, in the Alzheimer literature, is a term of art meaning the capacity to sustain pathology without clinical expression. No study has shown that cortical immature neurons contribute to reserve in that second sense.

What has been measured about these cells in Alzheimer's disease is, as far as we have been able to determine, nothing. There is no published count of doublecortin-positive layer II neurons in Alzheimer cortex against age-matched control. There is no measurement of their maturation state by Braak stage. There is no demonstration that they are lost, that they fail to mature, that they mature prematurely, or that they are unaffected. The chain's assertion that the pathological microenvironment "impairs their ability to mature and integrate properly" is, in the strict sense, a hypothesis for which the relevant stain has never been run on the relevant tissue.

This is worth stating plainly because it is the single most consequential gap in the whole chain, and because it is also the cheapest to close. The reagents are standard. Doublecortin immunohistochemistry on layer II of banked Alzheimer and control cortex, counted by region and stratified by Braak stage, with polysialylated neural cell adhesion molecule as a co-stain and NeuN to identify cells in transition, is an afternoon's protocol and a graduate student's project. That it has not been done is a fact about attention rather than about difficulty.

We note one methodological hazard that may partly explain the omission, because it bears on how the experiment should be designed. Doublecortin in the adult human cortex has been at the centre of a long and sometimes bitter dispute about adult hippocampal neurogenesis, in which the central contested issues were fixation interval, antibody specificity, and whether doublecortin-positive profiles in aged human tissue represent genuine immature neurons or artefact. Any study of layer II immature neurons in Alzheimer tissue inherits that dispute in full, and will be judged by whether it controls for post-mortem interval and fixation in the way the hippocampal literature learned, painfully, to do. The honest framing is that the measurement is easy and the measurement done *credibly* is not.

A second design constraint follows from Section II. Because the population is sparse in rodents and dense in large gyrencephalic brains, and because its density varies by cortical area, any human study must specify the area and any animal study must justify the species. A negative result in mouse neocortex would be uninformative. A negative result in human layer II would be decisive.

IV. The Sentinel What the Disease-Associated State Actually Is

The microglial joint of the chain is the one that survives the audit substantially intact, and it is worth setting out precisely because its accuracy throws the inverted joints into relief.

The disease-associated microglial state was defined by single-cell transcriptomics of all immune populations in wild-type and Alzheimer-transgenic mouse brain, with immunohistochemical confirmation in mouse and human tissue showing these cells containing intracellular amyloid particles (Keren-Shaul and colleagues, 2017). The activation is a two-step program. The first step is triggering-receptor-expressed-on-myeloid-cells-2-independent and consists principally of the downregulation of homeostatic microglial checkpoint genes. The second step is TREM2-dependent and installs the fully phagocytic program. The authors' own framing of the state is important and is routinely reversed in secondary accounts: they describe a cell type with "the potential to restrict neurodegeneration."

The chain uses the two-step structure correctly and then adds a clause the primary paper does not support — that the state becomes, with time, "chronically dysfunctional" and converts to a

net-toxic, synapse-stripping, matrix-degrading phenotype. This may well be true; it is a widely held and seriously argued position in the microglial literature. But it is a separate claim requiring separate evidence, and in the chain it is smuggled in as though it were part of the transcriptomic definition. The distinction matters for the exercise joint, as Section XII shows, because if the disease-associated state is protective then an intervention that enhances it is doing something the chain cannot accommodate.

We also note, and set aside, a terminological artefact. A phrase sometimes appears in secondary accounts — "microglia-associated degeneration," rendered as an acronym and used interchangeably with the disease-associated microglial state — which has no referent in the primary literature. It is not a defined entity, not a measured state, and not a term any laboratory uses. We mention it only because its presence is diagnostic: a mechanism that has acquired a coinage nobody uses has passed through at least one retelling that was not checked against a source.

V. The Sugar — Three Codes on One Polymer, and Which of Them Compete

Here the audit produces its one genuinely constructive result, and it is a better finding than the claim it replaces.

Heparan sulfate is a linear sulfated polysaccharide displayed on core proteins at the neuronal and glial surface and throughout the extracellular matrix. Its sulfation is not uniform. As the chain is polymerised it is modified in patches by a committee of enzymes — the N-deacetylase/N-sulfotransferases that install N-sulfate, and the 2-O-, 6-O- and 3-O-sulfotransferases that decorate specific ring positions — so the finished polymer is a mosaic of differently sulfated domains. Proteins that bind it do not bind "heparan sulfate"; they bind particular patterns.

Reelin reads N-sulfation. Surface plasmon resonance shows full-length reelin binding heparan sulfate with high affinity, with a dissociation constant of 17 ± 5 nanomolar, and the COLBOS variant binding more tightly at 10 ± 2 nanomolar. Competition and glycan-array studies identify N-sulfation as the critical determinant. In cells, heparinase treatment or knockout of NDST1, the key N-sulfotransferase, significantly reduces reelin attachment to the surface; and in a split-luciferase assay, heparinase or added heparin reduces reelin-induced ApoER2 dimerisation, while N-desulfated heparin does not (Pan and colleagues, 2025). The sugar is therefore not a backdrop but a co-receptor: without the N-sulfated bed, the receptor does not dimerise and the signal does not start.

TREM2 reads 6-O-sulfation. The microglial receptor binds cell-surface heparan sulfate and forms binary complexes with it, with effective binding requiring a minimum chain length of

about ten saccharide units. Structure-function dissection across surface plasmon resonance, low-molecular-weight microarray screening and serial mutant cell-surface binding assays identifies 6-O-sulfation as the crucial determinant, with the iduronic acid residue contributing to a lesser extent — and, decisively for the present argument, finds that *N-sulfation and 2-O-sulfation are dispensable*. The same 6-O-sulfation is required for TREM2-mediated apolipoprotein E uptake by microglia (McMillan and colleagues, 2024).

Tau also reads 6-O-sulfation. Transcellular propagation of tau begins with internalisation of seeds, and that internalisation is mediated by cell-surface heparan sulfate proteoglycans (Holmes and colleagues, 2013). The dependence is specific: genetic or enzymatic removal of the 6-O-sulfotransferase reduces tau entry into human neurons and brain slices (Rauch and colleagues, 2018), and a systematic comparison across aggregating proteins found tau to demand a more precise glycosaminoglycan architecture — defined chain length and defined sulfate positions — than α -synuclein or amyloid- β , which enter more promiscuously (Stopschinski and colleagues, 2018).

Now set the three readings side by side, and the chain's framing collapses in an instructive way. The chain asserts a "cross-sulfation war" in which the microglial receptor and the protective ligand compete for the same sugar, with the microglia winning and reelin starved. But the two proteins read marks that the primary experiments explicitly show to be separable: reelin requires N-sulfation; TREM2 explicitly does not need it. There is no substrate competition between them, because they are not binding the same thing.

What the data support is a different and sharper contest. The mark the microglial sensor requires is the same mark the tau seed requires. The real competition on the polymer is between the cell that clears pathology and the species that propagates it, both reading 6-O-sulfation — while the protective ligand reads a letter neither of them needs. This has an immediate therapeutic consequence that the war framing obscures entirely. An agent that strips 6-O-sulfation to block tau entry would, by the same action, impair TREM2 binding and TREM2-dependent apolipoprotein E uptake, disabling the clearance arm in order to close the entry route. But it would spare the N-sulfated bed on which reelin's signal depends. The knife-edge is real; it simply lies in a different place from where the chain puts it, and the protective ligand turns out to be on the safe side of it rather than the casualty.

A caution is owed, and it is the reason this section claims less than it could. The codes are not perfectly orthogonal — tau's uptake draws on N-sulfation as well as 6-O-sulfation, so the shield's alphabet and the gate's alphabet share a letter even if the sensor's and the shield's do not. And the sulfation pattern is not fixed: the 3-O-sulfotransferase HS3ST2 is upregulated in the Alzheimer hippocampus, with its 3-O-sulfated product relocating from membrane to the interior of degenerating neurons (Sepulveda-Diaz and colleagues, 2015), so the code drifts with disease. What has never been done is the measurement that would settle the whole section: paired quantification of

N-sulfated against 6-O- and 3-O-sulfated domains across disease stage in the same tissue. Until that exists, the orthogonality is a property of binding assays rather than a demonstrated property of the diseased brain.

VI. The Stalk The First Inversion

The chain requires a route by which microglial proteases silence reelin, and it finds one in the O-linked sugar domain of ApoER2. The account given is architectural and intuitive: the heavily glycosylated domain forms a rigid, carbohydrate-coated stalk that projects the ligand-binding repeats outward into the matrix; the glycans shield the protein backbone from proteolysis, but a bare, non-glycosylated neck at the domain's boundary is vulnerable; matrix metalloproteinases released by activated microglia cut at that neck, shedding the entire binding region and leaving a useless stump; reelin can no longer dock; Disabled-1 phosphorylation halts.

The domain is real. ApoER2 is produced in multiple splice variants, and the O-linked sugar domain is encoded by exon 16. The experiment that establishes the domain's function, however, runs the other way.

In mice and cells expressing ApoER2 lacking exon 16, extracellular cleavage of the receptor was *reduced*, and consequently the γ -secretase-dependent release of the receptor's intracellular domain was prevented. Mice expressing the domain-deficient receptor showed *increased* ApoER2 abundance in brain, increased hippocampal spine density and increased glutamate-receptor abundance — with decreased synaptic efficacy. When a form lacking the domain was combined with an alternatively spliced cytoplasmic tail that promotes glutamate-receptor signalling, hippocampal long-term potentiation was enhanced. Reducing expression of the mutant allele so that receptor abundance matched wild-type normalised spine density, potentiation and cued fear learning (Wasser and colleagues, 2014).

The O-linked sugar domain is therefore a *substrate* for shedding, not a shield against it. Its presence promotes extracellular cleavage; its removal stabilises the receptor at the surface and raises its abundance. The chain's picture — a protective sugar stalk whose proteolytic loss disables the receptor — inverts the published relationship between the domain and the protease.

Several consequences follow, and they are not symmetrical in importance.

First, the specific mechanism the chain proposes for microglial silencing of reelin loses its molecular basis. If anything, a proteolytic environment acting on this domain should be expected to engage the receptor's normal regulated processing rather than to abolish its ligand binding, and the phenotype of a brain with less processing at this site is one of *more* receptor, not less.

Second, and more interesting, the Wasser result identifies receptor abundance as a quantity the brain evidently needs held within a range. Raising ApoER2 by blocking its cleavage produced more spines and more glutamate receptors but less efficient synapses, and normalising abundance normalised function. A mechanism that proposes to rescue reelin signalling by raising receptor availability is therefore working against a system that already regulates that variable, and should expect a non-monotonic dose-response.

Third, the chain is not wrong that reelin signalling fails in the disease, and we are not claiming otherwise. There are well-evidenced routes by which it does — competition for the receptor by apolipoprotein E, and in the $\epsilon 4$ carrier the intracellular sequestration of ApoER2 so that less receptor reaches the surface at all. Those routes are trafficking lesions and binding lesions. The chain chose a proteolytic lesion at a domain whose proteolysis does the opposite of what the chain needs, and the other routes, which would have served, are not mentioned.

VII. The Ligand What Reelin Does in Layer II, Which Is Not Simple

The chain assigns reelin a single role: it is the dormancy brake, the signal that holds the immature cell safely arrested until it is needed, and whose loss permits aberrant premature maturation or degeneration. The evidence for reelin's importance in the layer II of Alzheimer's disease is strong. The evidence for this particular job description is absent, and the evidence that does exist complicates it in a direction nobody expected.

What is established is that reelin signals through ApoER2 and the very-low-density lipoprotein receptor, inducing tyrosine phosphorylation of the adaptor Disabled-1, and that the pathway's loss is associated with tau hyperphosphorylation (Hiesberger and colleagues, 1999). It is established that reelin signalling through this route phosphorylates cofilin at serine 3 and stabilises actin, though this was shown in a developmental context and has not been demonstrated in adult disease (Chai and colleagues, 2009).

And it is established, in the strongest form the field has, that reelin signalling matters for human resilience. A man heterozygous for a rare *RELN* variant — H3447R, named COLBOS — remained cognitively intact until the age of sixty-seven despite carrying the PSEN1-E280A mutation that causes autosomal-dominant Alzheimer's disease. He had an extremely elevated amyloid plaque burden with *limited entorhinal tau tangle burden*. The variant is gain-of-function, showing stronger activation of its canonical target Disabled-1 and reduced tau phosphorylation in a knock-in mouse (Lopera and colleagues, 2023). This is a single case, and should be read as one; but it is the second ascertained case of extreme resilience to autosomal-dominant disease, and both cases converge on the same receptor system.

Two observations about this case constrain the chain. The first is anatomical: the resilience was expressed as preserved entorhinal cortex in the face of overwhelming amyloid. The protected cells were the reelin-expressing projection neurons of entorhinal layer II — the second population of Section II — not the immature reserve. The second is mechanistic: the published gain of function is on Disabled-1 activation. The subsequent demonstration that the variant binds N-sulfated heparan sulfate more tightly (Pan and colleagues, 2025) supplies a plausible structural basis for that gain, but the frequently repeated claim that the variant leaves affinity for ApoER2 and the very-low-density lipoprotein receptor unchanged is not something either paper establishes; neither reports a receptor-affinity comparison. It should not be asserted.

The complication arrives from the layer itself. In reelin-expressing projection neurons of the anterolateral entorhinal cortex in a rat model that replicates the disease's spatio-temporal cascade, reelin and intracellular amyloid- β engage in a direct protein-protein interaction at pre-plaque stages. When a viral vector delivering micro-RNA against reelin was used to lower reelin in those neurons, intracellular amyloid- β fell concomitantly across three levels of aggregation — monomers and dimers, prefibrils and protofibrils — without substantial change in amyloid precursor protein, and Bayesian estimation of mutual information showed levels of amyloid- β to be dependent on levels of reelin (Kobro-Flatmoen and colleagues, 2023). Across 25,548 neurons from 24 animals, *less reelin meant less intracellular amyloid*.

This does not make reelin harmful, and we are careful not to overstate it. The authors themselves read it alongside the established facts that reelin signalling downregulates tau phosphorylation and that oligomeric amyloid- β interferes with reelin signalling, and propose that the interaction is relevant to why this particular neuronal population is vulnerable to tangles. But it is flatly incompatible with the chain's picture of reelin as a simple protective brake whose restoration is unambiguously good. In the very population and the very layer the chain is about, the protein forms a complex with intracellular amyloid, and its level sets the amyloid's level.

The honest summary is that reelin in layer II has at least three relationships to the disease — a protective signalling arm through Disabled-1 and tau, a resilience arm demonstrated by a gain-of-function human variant, and an intracellular arm in which it co-determines the burden of the species it is supposed to protect against — and that no account which uses only the first of these is complete. The claim that reelin maintains immature neurons in dormancy is, separately, unsupported in either direction: we found no study measuring reelin signalling in doublecortin-positive layer II cells, and no study in which manipulating reelin changed their maturation state.

VIII. The Switch The Second and Principal Inversion

This is the joint on which the chain's therapeutic content rests, and it is the one most completely reversed.

What was established in 2010. Ephexin5, the product of *ARHGEF15*, was identified as a RhoA guanine-nucleotide exchange factor that negatively regulates excitatory synapse development — a developmental brake restraining synapse formation until it is lifted. The lifting is proteolytic: ephrin-B binding to the EphB receptor tyrosine kinase triggers phosphorylation of Ephexin5, its ubiquitination by the ligase Ube3A, and its degradation. Degradation of Ephexin5 then permits EphB-dependent excitatory synapse development (Margolis and colleagues, 2010). This is the finding the chain's "synapse repressor" language comes from, and as far as it goes it is correct.

What was established about amyloid. Amyloid- β oligomers bind the fibronectin repeats of EphB2 and trigger its degradation in the proteasome; knockdown of EphB2 reduces NMDA-receptor currents and impairs long-term potentiation in the dentate gyrus, while increasing EphB2 expression in the dentate gyrus of amyloid-precursor-protein transgenic mice reverses deficits in potentiation and memory (Cissé and colleagues, 2011). Separately, Ephexin5 expression is elevated in the hippocampi of human Alzheimer patients and of transgenic mice; removal of Ephexin5 eliminated hippocampal spine loss and rescued behavioural deficits in those mice; and selective shRNA reduction of Ephexin5 in the dentate gyrus of pre-symptomatic adolescent transgenic mice was sufficient to protect against later cognitive impairment (Sell, Schaffer and Margolis, 2017).

Taken together these are a coherent and genuinely impressive case, and they are the empirical heart of the chain. Amyloid removes the kinase; the brake accumulates; synapses are lost; removing the brake rescues.

What was established in 2025, and which the chain cites while stating backwards. Ephexin5 is not a RhoA-specific exchange factor. It activates both RhoA and Cdc42 in brain. Using live imaging of Förster-resonance-energy-transfer GTPase biosensors at single synapses, Ephexin5 was shown to regulate activity-dependent Cdc42 signalling but *not* RhoA signalling during plasticity. The selectivity for Cdc42 activation is regulated by tyrosine phosphorylation, which is itself regulated by neuronal activity. And — the finding that dismantles the chain's prescription — downstream of neuronal activity, Ephexin5 *positively* regulates synaptic growth and stabilisation, in contrast to its negative regulation of synapse density (Petshow and colleagues, 2025).

The direction is explicit in the experiments. Phospho-tyrosine Ephexin5 falls developmentally, and chemical long-term potentiation reduces it by roughly three-quarters within minutes; that reduction survives proteasome inhibition, so it is dephosphorylation and not degradation. The phosphomutant that cannot be phosphorylated — Y361F — *enhances* Cdc42 activation while leaving RhoA activation unchanged. Spines in Ephexin5-knockout neurons failed to sustain activity-driven growth, returning to baseline volume against roughly twofold sustained growth in wild-type;

re-expressing full-length Ephexin5 restored growth, while a catalytically dead exchange factor did not.

So: **dephosphorylation biases toward Cdc42 and growth. Phosphorylation biases toward RhoA.** The chain states the opposite — that activity and exercise *phosphorylate* Ephexin5 through Fyn and Src to flip it into a Cdc42 activator, and that the loss of that phosphorylation traps it in the RhoA state. Every clause of that is reversed.

The coincidence that makes the inversion consequential. The residue at which the substrate switch is thrown is Y361 — the same tyrosine, in the same conserved regulatory motif, whose phosphorylation was characterised in 2010 as the event that recruits Ube3A and consigns the protein to the proteasome (Margolis and colleagues, 2010; Petshow and colleagues, 2025). Phosphorylation at that residue therefore does two things simultaneously: it biases the exchange factor toward RhoA, and it marks the exchange factor for destruction. Dephosphorylation likewise does two: it releases the Cdc42-directed activity, and it protects the protein from turnover.

Abundance and activity are not independent variables in this system. They are two readings of one covalent modification. This has a consequence for every measurement in the field and for every intervention proposed on it, and the consequence is uncomfortable: the phenotype "Ephexin5 is elevated" is ambiguous on its own. A cell with elevated Ephexin5 may have lost the kinase input that marks the protein for destruction — in which case the accumulated pool is hypo-phosphorylated and, by the 2025 model, Cdc42-biased and growth-promoting. Or it may have gained a phosphorylation input not coupled to Ube3A recruitment — in which case the accumulated pool is RhoA-biased. These two states have opposite structural consequences, and the antibodies and assays that report "Ephexin5 levels" report total protein and cannot distinguish them.

The tension this creates in the disease model, stated plainly. The model's premise is that amyloid depletes EphB2. EphB2 is the kinase that phosphorylates Y361. Loss of the kinase should therefore *lower* the phosphorylated fraction — which neatly explains the accumulation, since the phospho-form is the degraded form, but which predicts a hypo-phosphorylated, Cdc42-biased, growth-promoting pool. The observed phenotype is spine loss. Something must restore the RhoA bias that the loss of EphB2 removes, or the sign of the model is wrong.

There is a candidate, and it deepens rather than resolves the problem. In the Tg2576 model, the ligase Ube3A itself is depleted — following phosphorylation by c-Abl after amyloid- β exposure — and that depletion precedes behavioural deficits and spine loss; loss of Ube3A triggers accumulation of both Arc and Ephexin5, with activation of RhoA, and restoring Ube3A blocks the pruning (Olabarria and colleagues, 2019). This supplies a route to accumulation that does not require Y361 phosphorylation at all: the protein builds up because its ligase is gone. But it leaves the two published observations in direct contradiction. Olabarria reports RhoA activation accompanying Ephexin5 accumulation under conditions of failed degradation; Petshow's rule

predicts that a pool accumulating through failed phosphorylation-dependent degradation should be hypo-phosphorylated and Cdc42-biased. Both cannot be straightforwardly true of the same pool.

A second candidate exists: protein kinase C epsilon phosphorylates and activates Ephexin5 in a manner that suppresses spines (Schaffer and colleagues, 2018). If a kinase other than EphB2 phosphorylates the regulatory residue without licensing Ube3A recruitment — because the ligase requires a coincident EphB2-dependent signal that the disease has removed — then the Alzheimer dendrite would hold a pool that is simultaneously abundant and RhoA-locked, which is exactly the state the model requires. That is a hypothesis. It has not been tested.

The measurement that settles it, and why it has not been made. Quantify the phospho-Y361 fraction of Ephexin5, not total Ephexin5, in human Alzheimer hippocampus stratified by Braak stage and in amyloid-bearing mouse models, with parallel pulldown measurement of active RhoA and active Cdc42 in the same tissue. If the phospho-fraction is elevated and Cdc42 activity is not, the disease model survives the revision intact and the therapeutic target becomes the responsible kinase. If the phospho-fraction is reduced and Cdc42 activity elevated, the model's sign is wrong, and Ephexin5 accumulation in disease is a growth-directed and plausibly compensatory response to synapse loss rather than its cause — in which case the rescue seen on genetic deletion would be the removal of a failed repair attempt whose collateral cost exceeds its benefit. No published dataset answers this. It requires no new reagents beyond a validated phospho-Y361 antibody, which does not exist.

Why the therapeutic prescription cannot survive unexamined. The chain, and the 2017 work it rests on, prescribe lowering Ephexin5. Two independent facts now bear against doing so systemically. The first is the growth arm: Ephexin5 is *required* for activity-driven spine growth and stabilisation, so depleting it removes a function the neuron needs. The second is human genetics. Heterozygous loss-of-function mutations in *ARHGEF15* cause autosomal-dominant hereditary cerebral small-vessel disease with severe osteoporosis and osteoporotic fracture, acting through RhoA/ROCK2 *inactivation* with cytoskeletal disorganisation in vascular smooth muscle and endothelial cells, and a transgenic mouse recapitulates both phenotypes (Ding and colleagues, 2023). Chronic systemic reduction of Ephexin5 is therefore a phenocopy of a human vasculopathy that itself causes vascular dementia — which is to say, the proposed treatment for one dementia is the known cause of another.

What replaces the prescription is narrower and harder. If the disease-relevant lesion is a switch stuck in the RhoA position rather than an excess of protein, then the object of intervention is the switch: an agent biasing substrate selection toward Cdc42 without depleting the enzyme — functionally, a small-molecule mimic of the Y361F state — or an agent restoring the activity-driven dephosphorylation that potentiation normally produces. Neither exists.

IX. The Phosphatase The Third Inversion, Which Is Self-Inflicted

The chain reaches for an explanation of why the switch should be stuck, and finds one in the striatal-enriched protein tyrosine phosphatase. The account runs: amyloid raises STEP; STEP dephosphorylates and inactivates Fyn and Src; with the Src-family kinases silenced, Ephexin5 can never undergo its activity-dependent phosphorylation flip and is trapped in its default, toxic, RhoA-activating state; STEP inhibitors such as TC-2153 restore the flip.

The premises about STEP are well supported. STEP61 levels rise progressively in the cortex of transgenic mice over the first year and are elevated in the prefrontal cortex of human Alzheimer brain; the increase is associated with greater STEP activity, dephosphorylation of the regulatory tyrosine of the NR2B subunit, and decreased surface NR1 and NR2B; in STEP-knockout cultures, amyloid treatment fails to internalise NMDA receptors; and the mechanism of accumulation involves the ubiquitin–proteasome system (Kurup and colleagues, 2010). The inhibitor is real: TC-2153 inhibits STEP with a half-maximal concentration of 24.6 nanomolar through a reversible covalent interaction with the catalytic cysteine, raises tyrosine phosphorylation of STEP substrates in cells, and improves cognitive function in triple-transgenic Alzheimer mice at six and twelve months — notably *without* changing amyloid or phospho-tau levels (Xu and colleagues, 2014).

The inversion here is not in the STEP literature. It is a contradiction the chain creates between two of its own joints, and it follows in one step.

STEP removes phosphate from tyrosines. Less Src-family kinase activity on Ephexin5 means a *lower* phospho-Y361 fraction. By the rule established in the experiment the chain itself cites, a lower phospho-Y361 fraction means a *Cdc42* bias — the growth-promoting state. So elevated STEP, acting through the mechanism the chain describes, should push Ephexin5 toward growth, not away from it. The chain asserts the opposite while invoking both findings on the same page.

There are two ways out, and both are more interesting than the chain's version.

The first is that STEP's relevant action here is not on Ephexin5's regulatory tyrosine at all. STEP's demonstrated substrates in this context are the NMDA-receptor subunit, ERK1/2, Pyk2 and the Src-family kinases; nobody has shown that STEP acts on Ephexin5, directly or indirectly, and nobody has measured Ephexin5 phosphorylation in a STEP-manipulated preparation. On this reading the STEP joint is not inverted but simply absent — a connecting sentence with no experiment behind it — and STEP's contribution to the disease runs through receptor internalisation, which is well evidenced and sufficient on its own.

The second is that the Y361 kinase in the diseased dendrite is not a Src-family kinase at all but protein kinase C epsilon (Schaffer and colleagues, 2018), which is a serine/threonine kinase and therefore wholly unaffected by a tyrosine phosphatase. On this reading STEP could silence Fyn and Src entirely without touching the switch, and the RhoA bias would be maintained by a kinase the chain never mentions.

Either resolution preserves STEP as a legitimate target while removing it from the Ephexin5 story. What cannot be preserved is the chain's version, in which a tyrosine phosphatase maintains a tyrosine-phosphorylation-dependent state by removing the phosphate that creates it.

X. The Engine Where the Mechanism Becomes Genuinely Degenerate

Below the switch, the chain descends into the actin machinery, and here it is substantially correct about the biochemistry and substantially too confident about its interpretation.

The biochemistry is canonical. Active Cdc42 engages p21-activated kinase, which activates LIM-domain kinase, which phosphorylates cofilin at serine 3; phosphorylated cofilin cannot bind F-actin and cannot sever it. Active RhoA engages Rho-associated kinase, which also activates LIM-domain kinase, which also phosphorylates cofilin at serine 3. Cdc42 additionally drives branched nucleation through WASP and WAVE complexes and the Arp2/3 machinery, pushing membrane outward; RhoA drives contractile structures that pull it inward. Cofilin, when active, severs filaments; the spine's volume is the running balance of severing and nucleation.

The difficulty, which the chain does not register, is that **both Rho-family arms terminate on the same residue in the same direction**. RhoA→ROCK→LIMK raises serine-3 phosphorylation. Cdc42→PAK→LIMK raises serine-3 phosphorylation. A measurement of phospho-cofilin therefore does not identify which arm produced it, and the structural outcomes of the two arms are opposite. This is a genuine degeneracy in the readout, and it is the reason the human literature on cofilin in Alzheimer's disease contains results pointing in both directions that are not straightforwardly reconcilable: signalling through LirB2 has been reported to enhance cofilin activation — that is, dephosphorylation — in human Alzheimer brain, while amyloid acting through ROCK has been reported to *increase* serine-3 phosphorylation in human Alzheimer cortex and to be necessary and sufficient for impairment. Both are human observations. They are measurements of the same residue in opposite directions.

A mechanism that proposes to read the state of the spine from phospho-cofilin is therefore reading a variable that two antagonistic pathways write to identically. The chain treats low phospho-cofilin as the disease state and high phospho-cofilin as the rescued state, which is one of the two available readings and is not the one the ROCK literature supports.

The cofilin–actin rod is on firmer ground and deserves its place in the account. Rods are bundled assemblies that require *active*, dephosphorylated cofilin, form in neurites under stress, are present in human Alzheimer brain and are most prominent in neurites contacting amyloid deposits (Minamide and colleagues, 2000; Bamburg and Bernstein, 2016). As a structure, the rod is an unambiguous marker of the dephosphorylated state in a defined compartment, which is precisely what a bulk phospho-cofilin measurement is not. If the field wants a readout of cofilin's disease state that is not degenerate, rod burden by compartment is a better candidate than phospho-fraction by homogenate.

One further caution on the chain's treatment of this layer. It proposes allosteric modulators of p21-activated kinase as a therapeutic class on the grounds that the cell requires localised, timed remodelling rather than global activation or inhibition. That reasoning is sound, and it applies with equal force one step up: it is the argument against depleting Ephexin5, which the same chain recommends.

XI. The Laminar Argument What Is Actually Layer-Specific

The chain closes its anatomical section with a layer-by-layer map: layer I as a synaptic killing zone where microglia cluster and shed receptors; layer II as the crucible where the immature reserve is locked down; layer III as the site of long-range disconnection; layer IV as a resilient sensory sanctuary; layer V as the tangle hotspot where cofilin rods dam the axons; layer VI as the final corticothalamic failure. Each is then mapped onto a clinical stage.

Some of this is well founded. The selective vulnerability of entorhinal layer II is among the most secure observations in the neuropathology of the disease, and the relative sparing of primary sensory cortex — including its granular layer IV — through early and moderate Braak stages is a real and long-recognised pattern that makes those regions useful internal controls. The vulnerability of long-projecting pyramidal neurons with high metabolic demand is a reasonable reading of a large literature.

What is not founded is the molecular specificity. The claim that layer I is where microglia release metalloproteinases that cleave the O-linked sugar domains of receptors is not a measurement; no study has quantified laminar metalloproteinase activity against receptor ectodomain shedding by layer, and Section VI has already shown that the proposed cleavage runs the wrong way. The claim that layer II features a "uniquely dense" matrix of N-sulfated heparan sulfate and full-length reelin is an inference from the fact that entorhinal layer II neurons express reelin; the sulfation-domain mapping by layer has not been done, and it is exactly the measurement Section V named as missing. The claim that cofilin–actin rods are a layer V phenomenon creating

axonal traffic jams extrapolates from the general finding that rods form in neurites near amyloid to a laminar specificity nobody has reported.

The deeper problem is one of attribution. Layer II's documented vulnerability belongs to the entorhinal reelin-expressing projection neurons. The chain's reserve belongs to the doublecortin-positive immature cells. The chain uses the first population's well-established fate as evidence for the second population's hypothesised fate, which is the Section II conflation returning in anatomical dress. When the chain says that layer II is destroyed and therefore the reserve is lost, it has demonstrated the first clause in one population and asserted the second in another.

There is, however, a genuinely interesting question underneath the confusion, and it survives the audit. The two populations do share an address. If cortical immature neurons sit in the same layer that is attacked earliest, then whatever destroys the mature cells of that layer is operating in the immediate neighbourhood of the reserve — and the reserve's response, whatever it is, has never been looked at. That is a reason to run the stain of Section III, and it is a better reason than the chain's, because it does not require assuming the answer.

XII. The Intervention The Fourth Inversion

The chain's final movement is therapeutic and is the reason the whole structure is attractive: every lesion it names is reversed by exercise. Exerkines shift microglia out of the disease-associated state and reduce neuroinflammation; the reduced protease burden lets full-length reelin persist; restored reelin drives the kinase cascade that flips the switch; immature neurons are recruited back into memory circuits; cognition recovers.

The couriers are real. Cathepsin B is a genuine myokine, secreted by muscle under exercise stress, elevated in muscle and plasma by running, capable as a recombinant protein of raising brain-derived neurotrophic factor and doublecortin in hippocampal progenitors; mice lacking it fail to gain either neurogenesis or spatial memory from running, and treadmill exercise raises plasma cathepsin B in monkeys and in people, where its change tracks with fitness and hippocampus-dependent memory (Moon and colleagues, 2016). Irisin is reduced in the hippocampus and cerebrospinal fluid of Alzheimer patients and models; knocking it down impairs potentiation and memory, boosting it rescues both in model mice, and blocking it — centrally or peripherally — abolishes the neuroprotective effect of exercise itself (Loureço and colleagues, 2019). Global deletion of *FNDC5*/irisin impairs cognition across exercise, ageing and Alzheimer settings, and raising circulating irisin by hepatic overexpression enriches it centrally and improves both deficit and pathology (Islam and colleagues, 2021).

None of that is in dispute here. What is in dispute is the single study the chain treats as its capstone, because in it the two key results run opposite to the chain's use of them.

Single-nucleus RNA sequencing was used to dissect the response to free-wheel running in the neurogenic stem-cell niche of the hippocampal dentate gyrus in male APP/PS1 transgenic mice. Transcriptomic responses to exercise differed between wild-type and Alzheimer animals and were most prominent in immature neurons; exercise restored the transcriptional profiles of a proportion of dysregulated genes in a cell-type-specific manner; a neurovascular-associated astrocyte subpopulation reduced in disease had its signature induced by exercise; oligodendrocyte progenitors had the highest proportion of recovered genes; and key findings were validated in a human dataset.

Exercise also enhanced the gene expression profile of disease-associated microglia (da Rocha and colleagues, 2025).

Two things follow.

First, the microglial direction is the reverse of the chain's. Exercise did not shift microglia away from the disease-associated state; it strengthened that state's expression profile. This is consistent with the original characterisation of those cells as potentially restricting neurodegeneration (Keren-Shaul and colleagues, 2017), and inconsistent with the chain's requirement that the state be the toxic agent whose suppression is the mechanism of benefit. If exercise works partly by enhancing the disease-associated program, then the chain's central antagonist is, at least in part, an ally — and the matrix-degradation, reelin-cleaving, receptor-shedding cascade that the chain attributes to those cells cannot be the route by which exercise helps, because exercise increases the cells' defining program rather than decreasing it.

Second, the neuronal population is the wrong one. The immature neurons whose profiles responded most prominently are the adult-born neuroblasts of the dentate subgranular zone — stem-cell-derived, postnatally generated, dividing progeny. They are not cortical immature neurons, which are prenatally generated and never divide, and which are in any case virtually absent from the rodent neocortex (La Rosa and colleagues, 2020). The study is a careful piece of work on hippocampal neurogenesis in a mouse. It is not evidence about a cortical reserve in a human, and the chain's use of it as such requires the Section II conflation to have already been made.

The broader point about exercise is one the trial literature has made repeatedly and that the chain does not accommodate. Exercise acts on the substrate and resilience of the brain — the vascular and metabolic terrain, the synaptic and neurogenic reserve — rather than on the proteostatic engine that generates amyloid and propagates tau. A therapy that improves tolerance of pathology without arresting its production should delay the clinical threshold in people who have not crossed it and do little for those who have. That is the observed pattern, and it makes exercise a resilience modifier that has been repeatedly miscast as a late disease-modifier. The chain's version, in which exercise reverses each molecular lesion in turn, is the miscasting in its most complete form.

XIII. What Survives

It is easy for an audit to read as demolition, so it is worth stating compactly what remains standing, because it is not nothing and in places it is better than what it replaces.

Standing intact. The disease-associated microglial state and its two-step, TREM2-gated structure. The depletion of EphB2 by amyloid- β oligomers and its bidirectional rescue. The elevation of Ephexin5 in human Alzheimer hippocampus and the benefit of its removal in amyloid-bearing mice. The elevation of STEP61 in human Alzheimer cortex and the cognitive benefit of its inhibition. The requirement of N-sulfated heparan sulfate for reelin-induced receptor dimerisation. The existence, distribution and species scaling of cortical immature neurons. The reality of the exerkine couriers. The RELN-COLBOS resilience case.

Standing in improved form. The sugar. The chain's substrate war between microglia and reelin is replaced by a sharper and better-evidenced structure: reelin reads N-sulfation, which TREM2 does not need; TREM2 reads 6-O-sulfation, which tau also requires. The contest on the polymer is between the clearing cell and the propagating seed, and the protective ligand reads a letter outside that contest. This changes the therapeutic calculus for sulfation-directed agents and does so in a direction that is, for once, encouraging.

Standing as an open and important question. Whether cortical immature neurons are affected in Alzheimer's disease at all. The chain assumes they are; nobody has looked. The experiment is cheap, the tissue exists, and the answer would either open a new cellular arm of the disease or close a speculation that has been circulating without evidence.

Not standing. The sugar stalk as a protective spacer whose proteolysis silences reelin. The phosphorylation-activates-Cdc42 direction of the Ephexin5 switch. The STEP-to-Ephexin5 link. Exercise as a suppressor of the disease-associated microglial state. Reelin as a simple dormancy brake on immature neurons. And the therapeutic prescription that follows from the inverted switch — lower Ephexin5 — which the growth arm and the *ARHGEF15* vasculopathy jointly forbid as a systemic strategy.

XIV. The Validity Ledger

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 structural; **Inference** where the connection is ours and untested.

Claim	Source	Grade	Note
Cortical immature neurons are prenatally generated, non-dividing, and persist in layer II expressing DCX and PSA-NCAM	La Rosa et al., 2020	M3	Twelve species; morphology and molecular profile well preserved
They are virtually absent from rodent neocortex; linear density rises with brain size	La Rosa et al., 2020	M3	The constraint that disqualifies mouse neocortical inference
Immature-reservoir and stem-cell neurogenesis vary inversely across mammals (trade-off)	Bonfanti et al., 2023	Review	Hypothesis, explicitly framed as such by its authors
cINs persist into advanced age though declining through juvenile stages	Ghibaudi et al., 2023	M4	Mouse piriform cortex
Human amygdala paralaminar immature excitatory neurons mature across adolescence and persist into old age	Sorrells et al., 2019	M2	Embryonic to adult human series plus snRNA-seq
A homologous mouse paralaminar region exists; neurons embryonically born, maturing P21–P35	Alderman et al., 2023	M4	Establishes cross-species conservation of the phenomenon
cINs have never been examined in Alzheimer's disease	—	Absent	No count, no staging, no maturation measure. §III names the experiment
DAM activation is a two-step program, TREM2-independent then TREM2-dependent	Keren-Shaul et al., 2017	M3 / M2	Mouse scRNA-seq; human IHC confirmation
The DAM state is described by its discoverers as potentially restricting neurodegeneration	Keren-Shaul et al., 2017	M3	The chain's "chronically dysfunctional" clause is a separate claim
Reelin binds HS at K_D 17 ± 5 nM; COLBOS at 10 ± 2 nM; N-sulfation critical	Pan et al., 2025	M5	SPR, glycan array, NDST1-KO, heparinase; N-desulfated heparin does not block
HS is a co-receptor for reelin-induced ApoER2 dimerisation	Pan et al., 2025	M5	Split-luciferase; the functional step

Claim	Source	Grade	Note
TREM2 binds HS requiring 6-O-sulfation; N-sulfation dispensable; ≥ 10 saccharides	McMillan et al., 2024	M5	Also required for TREM2-mediated ApoE3 uptake
Tau internalisation requires 6-O-sulfation on HSPGs	Rauch et al., 2018	M5	Human neurons and brain slices
Tau demands more precise GAG architecture than α-synuclein or Aβ	Stopschinski et al., 2018	M5	Basis for selectivity being chemically real
HS3ST2 is upregulated in Alzheimer hippocampus; 3-O-sulfated product internalises	Sepulveda-Diaz et al., 2015	M2	The code drifts with disease
Reelin and TREM2 read non-overlapping sulfation marks; the real contest is TREM2 vs tau	This paper, from Pan 2025 + McMillan 2024 + Rauch 2018	Inference	Verifiable from the three sources; replaces the "cross-sulfation war"
ApoER2 O-linked sugar domain is encoded by exon 16; its deletion reduces extracellular cleavage and raises receptor abundance, spine density and GluR content	Wasser et al., 2014	M3	Inverts the chain's protective-spacer account
Reelin $\rightarrow$ ApoER2/VLDLR $\rightarrow$ Dab1; loss hyperphosphorylates tau	Hiesberger et al., 1999	M4	The protective arm's founding result
Reelin/ApoER2/Dab1 phosphorylates cofilin Ser3 and stabilises actin	Chai et al., 2009	M4	Developmental; not shown in adult disease
RELN-COLBOS (H3447R) gain-of-function confers resilience to ADAD; high plaque, limited entorhinal tangle	Lopera et al., 2023	M2	n = 1 index case; stronger Dab1 activation; knock-in mouse
COLBOS does not alter affinity for ApoER2/VLDLR	—	Unsupported	Neither Lopera 2023 nor Pan 2025 reports a receptor-affinity comparison
Lowering reelin in aLEC layer II neurons lowers intracellular Aβ across three aggregation levels	Kobro-Flatmoen et al., 2023	M4	25,548 neurons, 24 animals; APP unchanged; complicates the guardian reading

Claim	Source	Grade	Note
Reelin maintains cIN dormancy	—	Absent	No study measures reelin signalling in DCX+ layer II cells
EphB-mediated phosphorylation of Ephexin5 recruits Ube3A for proteasomal degradation	Margolis et al., 2010	M4	Biochemistry with ligase identification
Aβ binds EphB2 fibronectin repeats, triggers proteasomal degradation; restoring EphB2 rescues	Cissé et al., 2011	M3	Bidirectional manipulation in vivo
Ephexin5 elevated in human AD hippocampus; its removal eliminates spine loss and rescues behaviour in hAPP mice	Sell et al., 2017	M2 / M3	Human expression single report; mouse rescue robust
Ephexin5 activates both RhoA and Cdc42; selectivity set by tyrosine phosphorylation; Y361F enhances Cdc42	Petshow et al., 2025	M4	FRET biosensors at single spines; cLTP cuts phospho-Y361 ~75% in minutes
Ephexin5 is required for activity-driven spine growth; catalytically dead protein does not rescue	Petshow et al., 2025	M4	The growth arm that forbids depletion
Ephexin5 is necessary for site-specific new spine formation as well as a brake on outgrowth	Hamilton et al., 2017	M4	The dual role, established before the switch was known
The residue that selects substrate is the residue that licenses destruction	Margolis 2010 + Petshow 2025	Observation	Abundance and activity are one variable, not two
Ube3A is depleted in Tg2576 via c-Abl; loss accumulates Ephexin5 and activates RhoA	Olabarria et al., 2019	M4	In tension with Petshow's rule; §VIII states the contradiction
PKCϵ phosphorylates and activates Ephexin5, suppressing spines	Schaffer et al., 2018	M4	Candidate for the missing RhoA bias; a serine/threonine kinase

Claim	Source	Grade	Note
ARHGEF15 heterozygous LOF causes dominant cerebral small-vessel disease with osteoporotic fracture, via RhoA/ROCK2 inactivation	Ding et al., 2023	M1	Two families, two sporadic cases, transgenic mouse
STEP61 elevated in human AD prefrontal cortex; dephosphorylates NR2B pTyr1472; drives receptor internalisation	Kurup et al., 2010	M2 / M3	Human tissue plus Tg2576 time course
TC-2153 inhibits STEP (IC50 24.6 nM) and improves cognition in 3xTg-AD without changing Aβ or p-tau	Xu et al., 2014	M3	Target validation; note the pathology-independence
STEP traps Ephexin5 in the RhoA state	—	Contradicted	A tyrosine phosphatase lowers pY361, which by Petshow 2025 biases to Cdc42
Both RhoA→ROCK→LIMK and Cdc42→PAK→LIMK raise cofilin pSer3, with opposite structural outcomes	Canonical	M5	The degeneracy of §X; phospho-cofilin does not identify its source
Cofilin–actin rods require active (dephosphorylated) cofilin and occur in human AD brain	Minamide et al., 2000; Bamburg and Bernstein, 2016	M2 / M4	Most prominent in neurites contacting amyloid
Cathepsin B is a genuine exercise myokine on the neurogenic pathway	Moon et al., 2016	M3 / M2	Causal in mice; plasma correlate in monkeys and people
Irisin is required for exercise's cognitive benefit and rescues memory in AD models	Lourenço et al., 2019; Islam et al., 2021	M3	Blocking irisin abolishes exercise's benefit
Exercise enhanced the DAM gene-expression profile; responses most prominent in dentate immature neurons	da Rocha et al., 2025	M3	Inverts the chain's microglial direction; wrong neuronal population
Exercise reverses the molecular lesions of the chain in cortical layer II	—	Unsupported	No measurement in cortex, in cINs, or in a species that has them
All four inversions run toward a tidier mechanism	This paper	Observation	§XVII; a claim about synthesis, not about biology

Four entries in this table are marked **Absent**, **Unsupported** or **Contradicted** and name claims the chain treats as established. Two are the paper's own inferences and are labelled as such. These six are the first things a reader should attack.

XV. Predictions and Falsification

The audit is only worth making if it generates measurements. Six follow, ordered by how much they would change.

1. The phospho-Y361 fraction of Ephexin5 is elevated relative to total Ephexin5 in Alzheimer hippocampus, with active RhoA elevated and active Cdc42 unchanged or reduced in the same tissue. This is the decisive measurement for the whole switch. *Falsified by* a reduced or unchanged phospho-fraction with elevated total protein and elevated active Cdc42 — which would invert the sign of the synaptic model and recast Ephexin5 accumulation as a failed compensatory response rather than an executioner. Requires one validated phospho-Y361 antibody, which does not exist. Everything else on this list is cheaper; nothing else is as informative.

2. Doublecortin-positive layer II neurons are reduced, or show altered maturation stage, in Alzheimer cortex relative to age-matched control, stratified by Braak stage. *Falsified by* no difference, which would remove cortical immature neurons from the disease's cellular account and close a speculation that currently circulates without evidence. Must control post-mortem interval and fixation to the standard the adult-neurogenesis dispute established, and must specify cortical area.

3. In staged human entorhinal-hippocampal tissue, N-sulfated heparan-sulfate domains decline while 6-O- and 3-O-sulfated domains rise, in step with tau progression. *Falsified by* a stable code, which would remove the drift limb and leave the sulfation argument as a property of binding assays only. This is the measurement that makes or breaks Section V's therapeutic corollary.

4. ApoER2 ectodomain shedding in Alzheimer cortex is increased, not decreased, relative to control, and tracks with microglial protease burden by layer. The chain predicts loss of surface receptor by cleavage; Wasser predicts that engaging this processing route lowers, not raises, total receptor. *Falsified by* reduced shedding with reduced surface receptor, which would mean the lesion is in synthesis or trafficking rather than in proteolysis — and would point back toward the ApoE-dependent trafficking routes the chain does not use.

5. Exercise in an animal model that actually possesses neocortical cortical immature neurons alters their number or maturation state. No rodent study can test this. It requires a gyrencephalic species, and it is the only design that would convert the exercise

claim from analogy into evidence. *Falsified by* no change, which would confine exercise's cellular benefit to the hippocampal niche where it has been demonstrated.

6. Cofilin–actin rod burden, scored by compartment, separates Alzheimer from control tissue more cleanly than bulk phospho-cofilin fraction. This is a methodological prediction and follows directly from the degeneracy of Section X. *Falsified by* equivalent discrimination, which would mean the two Rho-family arms are not in fact co-active in the same tissue and the degeneracy is theoretical rather than practical.

XVI. Therapeutic Corollaries

Each inversion changes a prescription, and in three cases it reverses one.

On the exchange factor, do not deplete. The chain's prescription — lower Ephexin5 — is forbidden twice over: by the growth arm, since the protein is required for activity-driven spine growth and a catalytically dead version does not rescue; and by human genetics, since heterozygous loss of function produces cerebral small-vessel disease with osteoporotic fracture through RhoA/ROCK2 inactivation. A systemic Ephexin5-lowering agent would treat one dementia by causing another. The target, if the switch model survives measurement 1, is the *state* of the switch rather than the quantity of the protein: an agent biasing substrate selection toward Cdc42 without depletion — a pharmacological mimic of the Y361F state — or one restoring the activity-driven dephosphorylation that potentiation normally produces. Both would require structural work on the interface the phosphomutant studies have now localised. Neuron-restricted targeted degradation solves the vascular problem and not the intraneuronal one, because it still removes the protein rather than biasing it.

On the sugar, spare the N-sulfated bed and think carefully before stripping the 6-O. The corrected orthogonality makes the design problem more tractable than the war framing implied, but it relocates the hazard. An agent removing 6-O-sulfation to deny tau its portal would simultaneously deny TREM2 its ligand and impair TREM2-dependent apolipoprotein E uptake — disabling the clearing cell to close the entry route. It would, however, leave reelin's N-sulfated bed intact. The target is therefore sulfation-domain selectivity with an explicit accounting of the microglial cost, and the demonstration that a single sulfotransferase can be silenced to reverse tau phosphorylation without catastrophe is the encouraging precedent.

On the phosphatase, keep the target and drop the rationale. STEP inhibition is well supported on its own evidence — human tissue elevation, a characterised inhibitor, cognitive benefit in a model, notably without change in amyloid or phospho-tau. None of that requires the Ephexin5 link, which is contradicted. Retaining the link would predict that STEP inhibition biases the exchange factor toward RhoA, which is the direction the chain is trying to avoid.

On the kinase, PAK modulation is right for the reason the chain gives and should be applied one level up. The argument that global activation or inhibition backfires because the cell needs localised, timed remodelling is correct. It is also the argument against depleting Ephexin5, and the chain does not notice that it has made it.

On exercise, prescribe it for what it does. It is a real and plural modifier of the brain's resilience — vascular, metabolic, neurogenic — acting through identified couriers, whose benefit is banked early and spent late. It is not shown to reverse the molecular lesions catalogued here, and in the one transcriptomic study that bears on it, it moved the microglial state in the direction opposite to the one the chain requires. The clinician's task is to prescribe it as reserve-building for the well and function-preserving for the sick, and to resist selling it as disease modification. The scientist's task is the preclinical-window trial the field has avoided.

XVII. Coda The Direction of the Errors

It would be a comfortable conclusion that the chain is simply sloppy, and an uncomfortable one that it is not. The chain is not sloppy. Its sources are real, recent, well-chosen and in several cases excellent; whoever assembled it had read widely and had identified the genuinely important papers in five separate literatures. The failure is not of diligence but of direction, and the direction has a pattern.

Consider the four inversions together. The sugar stalk was turned from a shedding substrate into a protective shield — which gives the microglia a weapon and the neuron a casualty. The Ephexin5 switch was turned from dephosphorylation-activates-growth into phosphorylation-activates-growth — which lets the loss of a kinase be the lesion and makes the villain a brake rather than a required participant. The phosphatase was recruited to lock the switch, supplying a named enzyme and a named drug where the mechanism had only a gap. And exercise was made to suppress the microglial state rather than to enhance it — which closes the loop and makes the intervention complete.

Each inversion makes the mechanism tidier. Each supplies a cleaner villain, a more complete rescue, or a drug where there had been an absence. Not one of them makes the story more complicated, more contingent, or less actionable. Errors that arise from carelessness are randomly signed; these are not. They are the errors a mechanism makes when it is being built toward a conclusion rather than from a set of measurements, and the force doing the building is not dishonesty but narrative pressure — the same pressure that makes a connecting sentence feel like an inference rather than an assumption.

There is a structural reason this particular chain was vulnerable. It spans five literatures — comparative neuroanatomy, microglial transcriptomics, glycobiology, Rho-family signalling, and

exercise physiology — and no reviewer reads all five in the original. Each joint is checkable only by someone who will not be asked to check it. A glycobiochemist reading the sulfation section would have caught the orthogonality in an afternoon; a Rho-family specialist would have caught the switch direction immediately. Neither would have been sent the whole chain, because the whole chain is nobody's field.

The correctives are unglamorous and they are the ones this audit used. Read the experiment and not the introduction that cites it. Ask in which direction the measurement went, not merely whether the two molecules are connected. Check whether the experimental animal possesses the structure under discussion. And treat a mechanism that has acquired a vocabulary nobody uses as having passed through at least one retelling that was never checked.

What remains, after all of it, is a set of real cells in a real layer that nobody has examined in the disease, a sugar whose three readings are more separable than anyone claimed, a switch whose published direction forbids the treatment built on it, and a measurement that would settle the largest of these and requires one antibody that has not been made. That is a less satisfying structure than the chain. It is the one the evidence supports, and every part of it is testable.

References

- Alderman PJ, Saxon D, Torrijos-Saiz LI, Sharief M, Page CE, Baroudi JK, Biagiotti SW, Butyrkin VA, Melamed A, Kuo CT, Vicini S, García-Verdugo JM, Herranz-Pérez V, Corbin JG, Sorrells SF. Delayed maturation and migration of excitatory neurons in the juvenile mouse paralaminar amygdala. *Neuron*. 2023;112(4):574–592.e10. doi:10.1016/j.neuron.2023.11.010
- Bamburg JR, Bernstein BW. Actin dynamics and cofilin-actin rods in Alzheimer disease. *Cytoskeleton*. 2016;73(9):477–497.
- Bonfanti L, La Rosa C, Ghibaudi M, Sherwood CC. Adult neurogenesis and "immature" neurons in mammals: an evolutionary trade-off in plasticity? *Brain Structure and Function*. 2023;229(8):1775–1793. doi:10.1007/s00429-023-02717-9
- 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. *The Journal of Neuroscience*. 2009;29(1):288–299.
- 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. doi:10.1038/nature09635
- da Rocha JF, Lance ML, Luo R, Schlachter P, Moreira L, Iqbal MA, Kuhn P, Gardner RS, Valaris S, Islam MR, Gassner GM, Mazuera S, Healy K, Shastri S, Hibbert NB, Moran-Figueroa KV, Haley EB, Pfeiffer RD, Aygar S, Kastanenka KV, Brase L, Harari O, Benitez BA, Tucker NR, Wrann CD. Protective exercise responses in the dentate gyrus of Alzheimer's disease mouse model revealed with single-nucleus RNA-sequencing. *Nature Neuroscience*. 2025;28(7):1546–1561. doi:10.1038/s41593-025-01971-w
- 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 Neuropathologica*. 2023;145(5):681–705. doi:10.1007/s00401-023-02560-6
- Ghibaudi M, Marchetti N, Vergnano E, La Rosa C, Benedetti B, Couillard-Despres S, Farioli-Vecchioli S, Bonfanti L. Age-related changes in layer II immature neurons of the murine piriform cortex. *Frontiers in Cellular Neuroscience*. 2023;17:1205173. doi:10.3389/fncel.2023.1205173
- 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. *Molecular and Cellular Neurosciences*. 2017;80:66–74. 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.
- Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proceedings of the National Academy of Sciences*. 2013;110(33):E3138–E3147.
- Islam MR, Valaris S, Young MF, Haley EB, Luo R, Bond SF, Mazuera S, Kitchen RR, Caldarone BJ, Bettio LEB, Christie BR, Schmider AB, Soberman RJ, Besnard A, Jedrychowski MP, Kim H, Tu H, Kim E, Choi SH,

- Tanzi RE, Spiegelman BM, Wrann CD. Exercise hormone irisin is a critical regulator of cognitive function. *Nature Metabolism*. 2021;3(8):1058–1070.
- Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. 2017;169(7):1276–1290.e17. doi:10.1016/j.cell.2017.05.018
- Kobro-Flatmoen A, Battistin C, Nair RR, Bjorkli C, Skender B, Kentros C, Gouras G, Witter MP. Lowering levels of reelin in entorhinal cortex layer II-neurons results in lowered levels of intracellular amyloid- β . *Brain Communications*. 2023;5(2):fcad115. doi:10.1093/braincomms/fcad115
- Kurup P, Zhang Y, Xu J, Venkitaramani DV, Haroutunian V, Greengard P, Nairn AC, Lombroso PJ. A β -mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *The Journal of Neuroscience*. 2010;30(17):5948–5957. doi:10.1523/JNEUROSCI.0157-10.2010
- La Rosa C, Cavallo F, Pecora A, Chincari M, Ala U, Faulkes CG, Nacher J, Cozzi B, Sherwood CC, Amrein I, Bonfanti L. Phylogenetic variation in cortical layer II immature neuron reservoir of mammals. *eLife*. 2020;9:e55456. doi:10.7554/eLife.55456
- Lopera F, Marino C, Chandras AS, O'Hare M, Villalba-Moreno ND, Aguillon D, Baena A, Sanchez JS, Vila-Castelar C, Ramirez Gomez L, et al. Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man. *Nature Medicine*. 2023;29(5):1243–1252. doi:10.1038/s41591-023-02318-3
- Lourenço MV, Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, Gonçalves RA, Clarke JR, Beckman D, Staniszewski A, Berman H, Guerra LA, Forny-Germano L, Meier S, Wilcock DM, de Souza JM, Alves-Leon S, Prado VF, Prado MAM, Abisambra JF, Tovar-Moll F, Mattos P, Arancio O, Ferreira ST, De Felice FG. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. *Nature Medicine*. 2019;25(1):165–175.
- 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. doi:10.1016/j.cell.2010.09.038
- McMillan IO, Liang L, Su G, Song X, Drago K, Yang H, Alvarez C, Sood A, Gibson J, Woods RJ, Wang C, Liu J, Zhang F, Brett TJ, Wang L. TREM2 on microglia cell surface binds to and forms functional binary complexes with heparan sulfate modified with 6-O-sulfation and iduronic acid. *The Journal of Biological Chemistry*. 2024;300(9):107691. doi:10.1016/j.jbc.2024.107691
- Minamide LS, Striegl AM, Boyle JA, Meberg PJ, Bamberg JR. Neurodegenerative stimuli induce persistent ADF/cofilin-actin rods that disrupt distal neurite function. *Nature Cell Biology*. 2000;2(9):628–636.
- Moon HY, Becke A, Berron D, Becker B, Sah N, Benoni G, Janke E, Lubejko ST, Greig NH, Mattison JA, Duzel E, van Praag H. Running-induced systemic cathepsin B secretion is associated with memory function. *Cell Metabolism*. 2016;24(2):332–340.
- Olabarria M, Pasini S, Corona C, Robador P, Song C, Patel H, Lefort R. Dysfunction of the ubiquitin ligase E3A Ube3A/E6-AP contributes to synaptic pathology in Alzheimer's disease. *Communications Biology*. 2019;2:111. doi:10.1038/s42003-019-0350-5
- 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. *Journal of the American Chemical Society*. 2025;147(51):46773–46779. doi:10.1021/jacs.5c15573

- 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. *Science Advances*. 2025;11(13):eadp5782. doi:10.1126/sciadv.adp5782
- Rauch JN, Chen JJ, Sorum AW, Miller GM, Sharf T, See SK, Hsieh-Wilson LC, Kampmann M, Kosik KS. Tau internalization is regulated by 6-O sulfation on heparan sulfate proteoglycans (HSPGs). *Scientific Reports*. 2018;8(1):6382. doi:10.1038/s41598-018-24904-z
- Schaffer TB, Smith JE, Cook EK, Phan T, Margolis SS. PKC ϵ inhibits neuronal dendritic spine development through dual phosphorylation of Ephexin5. *Cell Reports*. 2018;25(10):2470–2483.e8.
- Sell GL, Schaffer TB, Margolis SS. Reducing expression of synapse-restricting protein Ephexin5 ameliorates Alzheimer's-like impairment in mice. *The Journal of Clinical Investigation*. 2017;127(5):1646–1650. doi:10.1172/JCI85504
- Sepulveda-Diaz JE, Alavi Naini SM, Huynh MB, Ouidja MO, Yanicostas C, Chantepie S, Villares J, Lamari F, Jospin E, van Kuppevelt TH, Mensah-Nyagan AG, Raisman-Vozari R, Soussi-Yanicostas N, Papy-Garcia D. HS3ST2 expression is critical for the abnormal phosphorylation of tau in Alzheimer's disease-related tau pathology. *Brain*. 2015;138(Pt 5):1339–1354.
- Sorrells SF, Paredes MF, Velmeshev D, Herranz-Pérez V, Sandoval K, Mayer S, Chang EF, Insausti R, Kriegstein AR, Rubenstein JL, Manuel Garcia-Verdugo J, Huang EJ, Alvarez-Buylla A. Immature excitatory neurons develop during adolescence in the human amygdala. *Nature Communications*. 2019;10(1):2748. doi:10.1038/s41467-019-10765-1
- Stopschinski BE, Holmes BB, Miller GM, Manon VA, Vaquer-Alicea J, Prueitt WL, Hsieh-Wilson LC, Diamond MI. Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau versus α -synuclein and β -amyloid aggregates. *The Journal of Biological Chemistry*. 2018;293(27):10826–10840.
- Wasser CR, Masiulis I, Durakoglugil MS, Lane-Donovan C, Xian X, Beffert U, Agarwala A, Hammer RE, Herz J. Differential splicing and glycosylation of Apoer2 alters synaptic plasticity and fear learning. *Science Signaling*. 2014;7(353):ra113. doi:10.1126/scisignal.2005438
- Xu J, Chatterjee M, Baguley TD, Brouillette J, Kurup P, Ghosh D, Kanyo J, Zhang Y, Seyb K, Ononenyi C, Foscue E, Anderson GM, Gresack J, Cuny GD, Glicksman MA, Greengard P, Lam TT, Tautz L, Nairn AC, Ellman JA, Lombroso PJ. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. *PLoS Biology*. 2014;12(8):e1001923. doi:10.1371/journal.pbio.1001923