

JACOB'S MODEL - PAPER IV

APOLIPOPROTEIN-DEFINED LIPID CARGO IN RUBELLA AND DENGUE

A Falsifiable Host-State Model Linking ApoE/ApoA Particle Routing
to Lysosomal Load, NPC2-Dependent Cholesterol Availability, and Viral Biology

Jacob Boatman and Scott Boatman

Jacob's Voice

11 September 2026

Final definitive Paper IV - integrated evidence record, host-state model, and falsification program

Jacob would like to give credit to God for his amazing abilities.

Scope and Evidence Rule

This paper asks whether apolipoprotein-defined particle composition, receptor choice, and intracellular recycling modify the amount and fate of lipid cargo entering RUBV-remodeled cells, and whether that upstream host state changes the pressure placed on the downstream NPC2-centered cholesterol-throughput system defined in Paper I. Dengue virus (DENV) is used only where it supplies direct named-apolipoprotein or cholesterol-routing experiments that sharpen the experimental design.

This paper does not claim that APOE or APOA genes cause autism, that APOE genotype is a demonstrated RUBV susceptibility factor, that historical LDL/VLDL/HDL fractions identify a specific modern apolipoprotein, or that a direct RUBV-ApoE/ApoA interaction has already been demonstrated. Every claim is separated into direct RUBV evidence, direct DENV evidence, direct host biology, comparator evidence, hypothesis, or not yet shown.

Author's Note - Origin and Governance of the Model

The model developed here originated within Jacob Boatman's broader Rubella research program. Jacob's organizing principle is that biological outcome can depend on movement, compartmental delivery, and availability rather than abundance alone. Paper IV applies that principle upstream of the Paper I NPC2 bottleneck: the composition and routing of an incoming lipoprotein particle may determine how much lipid is recycled, effluxed, or committed to lysosomal processing.

Scott Boatman serves as coauthor, research collaborator, and literature investigator. The evidence decides every arrow. Historical density-class data are not silently converted into named Apo identities, comparator-virus mechanisms are not converted into RUBV mechanisms, and baseline APOE isoform biology is not called RUBV susceptibility without a virus-by-host-state interaction.

Abstract

Human lipoprotein particles are structured cargo rather than interchangeable pools of cholesterol. Their apolipoprotein composition changes receptor use, endosomal sorting, recycling, efflux, and the fraction of particle lipid committed to lysosomal degradation. Historical RUBV experiments directly show that lipoprotein particle architecture and physical state matter: native LDL and VLDL each produced RUBV haemagglutination-inhibition titers of 160, extracted lipid fractions retained activity at 128, apo fractions fell to 15 and 10, sonication increased activity to 480 and 640, and simple lecithin dispersions containing cholesteryl oleate, triolein, or cholesterol were each below 5. These results establish a particle-state effect but do not identify ApoE or ApoA-I as the responsible RUBV-facing determinant.

Independent host-cell biology defines a mechanistically relevant routing fork. ApoE-containing particles can enter endosomes, while ApoE itself can be recycled/resecreted and ApoB/core lipid proceeds toward lysosomal degradation; HDL/ApoA-I can stimulate ApoE recycling and cholesterol efflux. RUBV also strongly induces CH25H in primary human endothelial cells, and independent glial studies show that 25-hydroxycholesterol can increase ABCA1 and ApoE-containing lipoprotein secretion while suppressing LDLR-dependent reuptake and changing cholesteryl-ester storage. The full RUBV → CH25H/25HC → ApoE/ABCA1/LDLR program has not been demonstrated in one system.

DENV provides direct named-apolipoprotein comparator biology that RUBV currently lacks. ApoA-I can enhance DENV attachment through SR-BI, DENV capsid/VLDL biology can involve ApoE, NS1 forms HDL/ApoA-I-containing complexes including patient-associated lipoprotein complexes, and DENV can increase cholesterol ingress through LDLR/NPC1L1-linked routes while depending on correct late-endosomal/lysosomal cholesterol movement. These results show that named apolipoproteins can become viral-interaction and routing determinants, but they are not RUBV proof.

Paper IV therefore proposes a host-state interaction rather than an Apo causation claim. ApoE/ApoA-defined particle routing may alter the upstream lipid load delivered to a maturing RUBV-remodeled endolysosomal system whose leading Paper I bottleneck is NPC2-mediated post-LAL cholesterol availability. The decisive result is not an APOE association. It is a reproducible RUBV x particle/APOE-state interaction that changes recycling versus lysosomal commitment, NPC2 availability or transfer, late-endosomal/lysosomal free-cholesterol residence, or ER arrival, with rescue or pathway-specific perturbation. Failure to detect such an interaction despite robust infection and sensitive flux measurements would substantially weaken the central Paper IV hypothesis.

1. Central Question

Does apolipoprotein-defined particle composition alter the amount, receptor route, and intracellular fate of lipid cargo entering RUBV-remodeled cells strongly enough to change the magnitude of the downstream cholesterol-availability phenotype?

The question is deliberately interaction-based. An APOE isoform can differ from another at baseline without validating Paper IV. A particle can change viral attachment without validating Paper IV. The hypothesis requires that RUBV and a defined host/particle state interact to change intracellular lipid routing or downstream cholesterol throughput.

2. What Is Already Established - and What Is Not

Evidence class	Established observation	Boundary
DIRECT RUBV	LDL/VLDL/HDL-associated historical activity; strong particle-state dependence; modified lysosomal CPVs; cargo-handling defects; P150N-ARL8A interaction; LIPA/CH25H/CLN8/CTSS remodeling; human astrocyte/microglial tropism.	No direct named ApoE/ApoA interaction or RUBV x APOE-state flux phenotype.
DIRECT DENV	ApoA-I/SR-BI attachment; ApoE/VLDL/capsid and NS1-lipoprotein biology; increased LDL/cholesterol ingress; requirement for proper late-endosomal/lysosomal cholesterol movement; NPC2 compartment changes.	Comparator mechanism, not RUBV mechanism.
DIRECT HOST	ApoE/remnant routing; ApoA-I-assisted ApoE recycling; 25HC regulation of ABCA1/ApoE/LDLR; APOE isoform-dependent lysosomal/cholesterol/pH phenotypes; LAL-NPC2/NPC1 flux biology.	Host capacity and baseline state only.
NOT YET SHOWN	Direct RUBV-ApoE/ApoA binding; RUBV-induced ApoE secretion/lipidation/recycling changes; RUBV x APOE isoform interaction; Apo-dependent change in RUBV NPC2/ER-arrival flux.	The decisive Paper IV gap.

3. Density Class Is Not Molecular Identity

Historical RUBV papers worked mainly with HDL, LDL, VLDL, alpha-lipoprotein, beta-lipoprotein, and pre-beta-lipoprotein. These are operational density/electrophoretic classes, not single apolipoproteins.

Modern lipoprotein biology makes the boundary unavoidable. ApoE can exchange among triglyceride-rich remnants and subsets of HDL, while ApoA-I dominates many HDL particles and ApoB remains with LDL/VLDL-derived particles. An old 'VLDL effect' cannot therefore be renamed an ApoE effect, and an old 'HDL effect' cannot be renamed an ApoA-I effect without composition data.

This uncertainty does not weaken the historical experiments. It shifts their strongest conclusion from named-apolipoprotein specificity to particle architecture and particle state.

4. Direct RUBV Lipoprotein Evidence: The Quantitative Particle-State Signature

Historical preparation	RUBV HI activity
Native LDL	160
Native VLDL	160
Extracted LDL lipid fraction	128
Extracted VLDL lipid fraction	128
apoLDL	15
apoVLDL	10
Sonicated LDL	480
Sonicated VLDL	640
Lecithin + cholesteryl oleate	<5

Historical preparation	RUBV HI activity
Lecithin + triolein	<5
Lecithin + cholesterol	<5

The pattern is mechanistically informative. Removing particle protein lowers the historical RUBV-facing activity sharply, yet extracted lipid fractions retain substantial activity. Sonication greatly increases activity. Simple neutral-lipid dispersions fail. Phospholipase A treatment also changed activity. The most defensible interpretation is therefore that particle organization, lipid/protein presentation, and physical state materially control the RUBV assay phenotype.

The assay used mildly acidic buffers around pH 6.0-6.2. Those values are assay chemistry and must not be translated into intracellular CPV or lysosomal pH.

5. RUBV Particle Effects Are State-Dependent, Not Concentration-Only

Historical RUBV lipoprotein activity varied with serum preparation, storage, freeze-thaw, incubation time, pH, and metabolic state of VLDL preparations. Such sensitivity is difficult to explain with a model based only on bulk cholesterol concentration.

The particle-state model predicts that changing surface accessibility, apolipoprotein occupancy, phospholipid presentation, or particle remodeling can change the virus-facing phenotype even when the nominal density class is unchanged. Direct named Apo attribution remains unresolved.

6. Direct RUBV Human Sterol-Routing Signal: CH25H

The Geyer primary human endothelial dataset contains a strong direct RUBV sterol-response signal. CH25H rises approximately 39.06-fold under the study's stringent filtered analysis, alongside LIPA induction, CLN8 suppression, and strong CTSS induction.

CH25H matters to Paper IV because its product, 25-hydroxycholesterol, is independently capable of altering cholesterol handling in glial systems: increasing ABCA1 and extracellular ApoE-containing lipoprotein output, suppressing LDLR-dependent reuptake, changing cholesterol synthesis, and increasing cholesteryl-ester storage.

The direct RUBV result is CH25H induction. The downstream 25HC → ApoE/ABCA1/LDLR biology is independent host evidence. A RUBV-driven ApoE program remains unmeasured until 25HC, ApoE secretion/lipidation, ABCA1, and LDLR are quantified in the same infected system.

7. The Host ApoE/ApoA Routing Fork

After uptake of ApoE-containing remnant/triglyceride-rich particles, endosomal sorting can separate exchangeable ApoE from the degradative fate of ApoB/core lipid. ApoE can be retained, recycled, and resecreted, while particle core lipid proceeds toward lysosomal processing.

HDL/ApoA-I can enter the same broad endosomal cholesterol-recycling system and stimulate ApoE recycling and cholesterol efflux. This creates a true upstream load-control point: the fraction of incoming lipid returned to recycling/efflux pathways need not equal the fraction committed to lysosomal degradation.

Paper IV therefore treats ApoE and ApoA-I not as simple serum biomarkers but as determinants of routing probability, particle-state behavior, and lysosomal load.

8. DENV Provides Direct Named-Apolipoprotein Comparator Biology

DENV supplies the molecular resolution absent from historical RUBV work. ApoA-I can enhance initial DENV attachment through SR-BI/SCARB1, and SR-BI knockdown blunts that enhancement. DENV NS1 forms stable HDL/ApoA-I-containing complexes, including infection-derived and patient-associated lipoprotein complexes.

ApoE also appears in DENV/VLDL/capsid and circulating NS1-lipoprotein biology. These experiments establish that named apolipoproteins can become direct viral-interaction molecules and can alter the physical state of viral proteins or particles.

The boundary is strict: a named-Apo effect in DENV does not establish the same binding partner, receptor, or mechanism for RUBV. Its value is experimental precedent and assay design.

9. DENV Is Also an Ingress-versus-Egress Comparator

DENV can increase surface LDLR and LDL uptake and can engage NPC1L1-linked cholesterol acquisition. Conversely, trapping cholesterol in late endosomes/lysosomes retards DENV trafficking or replication. Human DENV proteomics also places NPC2 in altered secretome and membrane-associated compartments, while direct NPC2 RNAi did not identify NPC2 as a significant dependency hit in the recovered screen.

This mixed pattern supports the central Paper IV/Paper I principle: localization and flux can matter more than simple abundance. It also argues against using any single Apo or NPC2 concentration as the final mechanistic endpoint.

10. Neural Cell Context Makes the Host-State Test Biologically Relevant

RUBV has direct human neural evidence in cell classes that dominate CNS lipid exchange. Historical fetal glial work shows strong astrocyte permissiveness, and modern human fetal-brain/organoid systems show predominant microglial infection with profound interferon responses in neighboring neurons and progenitors.

Astrocytes are major CNS ApoE producers, and microglia are both RUBV-relevant neural-immune cells and active lipid-handling cells. The appropriate test system is therefore not a serum lipid panel; it is a human astrocyte-microglia or microglia-containing neural system in which ApoE secretion, lipidation, uptake, recycling, and lysosomal flux can be resolved.

11. APOE Isoform Is a Host State, Not a Causal Claim

Independent human glial models show APOE isoform-dependent differences in lysosomal cholesterol handling, NPC-related biology, endolysosomal pH, and cholesterol efflux/lipidation. These studies did not contain RUBV.

The proper Paper IV inference is therefore interaction susceptibility, not APOE causation. If APOE2, APOE3, and APOE4 systems differ at baseline but RUBV produces the same proportional routing and flux effect in all three, APOE is baseline host biology rather than a RUBV modifier.

A host-state modifier is supported only if infection changes the magnitude, direction, or location of the phenotype differently across otherwise matched APOE states.

12. Paper IV Now Sits Upstream of an NPC2-Dominant Paper I Bottleneck

The leading Paper I phenotype is post-LAL and NPC2-dominant: LDL-derived CE is substantially hydrolyzed, free cholesterol is generated, but lysosomal free-cholesterol residence increases and ER arrival falls because NPC2 availability or transfer is inadequate.

Paper IV therefore no longer proposes ApoE/ApoA as the bottleneck itself. It proposes that ApoE/ApoA-defined routing changes the load placed on that bottleneck.

A particle state that increases lysosomal commitment could worsen free-cholesterol accumulation if NPC2 export is limiting. A state favoring recycling or efflux could reduce the downstream load. These are direct flux predictions, not assumptions.

13. Four-Gate Model with Paper IV as the Load-Control Layer

Gate	Mechanism	Paper IV role	Required readout
1. Lipoprotein input	Particle identity, receptor choice, recycling vs lysosomal commitment	Primary Paper IV layer	Defined-particle binding/uptake/recycling; LDLR/LRP1/SR-BI/ABCA1; ApoE/ApoA state
2. Soluble lysosomal cargo	CI-M6PR/GF2R, RUFY1, ARL8/HOPS; NPC2/LAL/SMPD1/FUCA1 delivery	Downstream processing capacity facing the incoming load	Lysosomal vs secreted cargo; receptor retrieval; ARL8/HOPS state
3. Deep lysosomal chemistry	pH, SM->ceramide, PG/BMP/LBPA, NPC2 transfer competence	Determines whether delivered cargo can handle the load	Calibrated pH; species-resolved membrane lipids; direct NPC2 transfer
4. Post-NPC1 transfer	Contact-site/ER delivery and SOAT	Defines true downstream availability	ORP1L/VAP/contact-site readouts;

Gate	Mechanism	Paper IV role	Required readout
	re-esterification		ER arrival; SOAT/CE formation

14. M6P Cargo and Apo-Defined Input Must Be Measured Together

Changing particle input without measuring soluble lysosomal cargo can misidentify the limiting step. NPC2, LAL, SMPD1/acid sphingomyelinase, and FUCA1 provide a mechanistically useful cargo panel because they span cholesterol transfer, CE/TG hydrolysis, SM-to-ceramide chemistry, and fucose-containing substrate turnover.

Apo-defined input can therefore be experimentally crossed with cargo availability. If an Apo state increases lipid delivery but all soluble cargo remains normal, the phenotype points toward load, chemistry, or post-NPC1 limitation. If the same Apo state changes cargo routing only during RUBV infection, the result supports an interaction between upstream particle state and the endolysosomal machinery.

15. pH and Membrane Chemistry Remain Separate Variables

Paper IV retains pH because lipoprotein handling, RUBV entry, LAL activity, and NPC2 transfer are pH-sensitive processes. It does not infer that APOE genotype determines lysosomal pH *in vivo*.

No calibrated absolute pH of an established RUBV-positive CPV has been recovered. LysoPrime Green is not a quantitative pH reporter, and acidification-inhibitor experiments establish dependence rather than a numeric luminal value.

SGMS1 work also separates pH from membrane chemistry. Sphingomyelin, ceramide, glycosphingolipids, and BMP/LBPA-related chemistry can change transfer competence even when acidity is held constant. Apo-state, pH, membrane lipid chemistry, and NPC2 transfer therefore require independent measurement.

16. Historical Whole-Cell Lipids Reinforce the Availability Principle

Historical RUBV-infected BHK21 cells showed nearly unchanged bulk free cholesterol, 5.6 in control versus 5.8 in infected cells, despite broad phospholipid remodeling. The finding directly demonstrates why a whole-cell cholesterol measurement can miss a compartmental trafficking phenotype.

The late RUBV-associated fucosylglyceride further shows that chemically specific lipid remodeling emerges during established infection. Its exact synthetic and degradative pathway remains unresolved and is not assigned here to a specific apolipoprotein, fucosidase, or glycosphingolipid class.

17. Stage Dependence Matters to a Host-State Interaction

Apo-defined input is unlikely to have a single effect across all infection stages. Early RUBV biology is dominated by particle interaction, entry, and low-pH membrane events. Established and late infection adds virus-modified endolysosomal replication compartments, cargo-routing changes, acidic-vesicle phenotypes, and membrane remodeling.

Stage	Primary Paper IV question
Entry / early hours	Does defined Apo/particle state change viral attachment, uptake, or receptor choice?
12-24 h establishment	Does Apo state change recycling versus lysosomal commitment as replication structures establish?
48-72 h maturation	Does host state change the magnitude of NPC2/cargo/pH/membrane-chemistry divergence?
Multi-day infection	Does Apo state change lysosomal FC residence, ER arrival, or persistent routing adaptation?
Persistent infection	Does the RUBV x host-state interaction persist after acute entry/interferon effects?

An attachment effect confined to early infection is biologically real but does not validate the Paper IV cholesterol-throughput mechanism unless downstream routing or flux also changes.

18. The Central Interaction Model

ApoE/ApoA particle state -> receptor/endosomal route -> recycling versus lysosomal commitment -> LAL-mediated CE hydrolysis -> FC generation -> NPC2/NPC1 export -> downstream availability.

RUBV can intersect this sequence at several experimentally distinct positions: historical interaction with structured lipoprotein particles; strong CH25H induction; infection of ApoE-relevant human glial cells; and remodeling of the endolysosomal system in which the downstream cholesterol bottleneck is proposed.

The specific Paper IV prediction is not that any one Apo state is intrinsically 'good' or 'bad.' It is that particle and host state alter the load-response relationship of the infected cell.

19. Decisive Experiment

- Use isogenic APOE2/APOE3/APOE4 human iPSC-derived astrocyte-microglia systems or an equally controlled human glial platform, with matched mock and active RUBV conditions.
- Use chemically defined ApoA-I/HDL and ApoE/remnant-particle conditions and independently quantify particle composition, binding, uptake, endosomal recycling, and commitment to lysosomal degradation.
- Measure RUBV burden and stage, CH25H/25HC, ApoE secretion/lipidation/particle size, ApoA-I internalization/recycling, LRP1, LDLR, SR-BI/SCARB1, and ABCA1.
- Trace particle-derived CE through lysosomal delivery, LAL hydrolysis, FC generation, late-endosomal/lysosomal residence, NPC2/NPC1 egress, ER arrival, and SOAT-dependent re-esterification.
- Measure NPC2, LAL, SMPD1, and FUCA1 in total cell, purified late-endosomal/lysosomal or CPV-enriched fractions where feasible, and extracellular medium.
- Measure CI-M6PR/IGF2R retrieval/recycling, RUFY1, ARL8A/B, VPS39/VPS41/HOPS, calibrated compartment pH, SM/ceramide/PG/BMP/LBPA, and direct NPC2 transfer.
- If NPC2/NPC1 appear normal while ER arrival remains low, add ORP1L/VAP and other contact-site readouts.
- Test interaction terms explicitly: particle state x RUBV and APOE genotype x RUBV. Baseline differences without an infection interaction do not validate Paper IV.
- Use targeted rescue/perturbation where feasible: ApoE isoform swap or rescue, ABCA1/LRP1/SR-BI modulation, CH25H/25HC manipulation, and downstream cargo/transfer rescue.

20. Predetermined Interpretation Matrix

Observed pattern	Interpretation
Apo/particle state changes uptake/recycling equally in mock and RUBV	Baseline host-routing effect; not a RUBV modifier.
RUBV disproportionately increases lysosomal load in one Apo state and NPC2 export becomes limiting	Supports upstream load modifier acting on the downstream NPC2 bottleneck.
Apo state changes NPC2/LAL/SMPD1/FUCA1 routing only during RUBV	Supports infection-dependent coupling between particle state and soluble-cargo availability.
Apo state changes viral attachment but LDL-CE-to-ER flux remains normal	Entry/attachment effect only; does not validate Paper IV throughput model.
NPC2/NPC1 remain intact but ER arrival differs by RUBV x Apo state	Post-NPC1/contact-site modifier favored.
CH25H/25HC changes with RUBV and ApoE/ABCA1/LDLR routing follows; targeted perturbation rescues flux	Strong support for the CH25H-linked upstream modifier branch.
No reproducible RUBV x Apo/particle interaction across routing or cholesterol flux	Central Paper IV host-modifier hypothesis substantially weakened.

21. What Would Move Paper IV to the Edge of Direct Mechanism

The strongest result would not be an association between APOE genotype and a clinical phenotype. It would be a controlled virus-by-host-state interaction in which a defined Apo/particle state changes a specific intracellular routing step during RUBV infection, that routing change alters the downstream NPC2/ER-arrival phenotype, and targeted perturbation or rescue reverses the effect.

A complete positive chain would therefore contain: defined particle state -> altered receptor/recycling or lysosomal commitment during RUBV -> altered lysosomal load -> altered NPC2 availability/transfer or downstream ER arrival -> rescue by changing the implicated Apo/receptor/CH25H or downstream trafficking node.

That experiment would convert Paper IV from convergent host-state biology into a direct RUBV modifier mechanism. Until then, APOE/ApoA remain experimentally compelling host-state candidates, not established RUBV susceptibility determinants.

22. Relationship to Papers I-III

Paper I defines the downstream cholesterol-throughput system and now places NPC2-mediated post-LAL availability as the leading bottleneck. Paper IV supplies the experimentally controllable input/load dimension acting on that system.

Paper II can use ApoE/ApoA only as a potential glial host-state modifier; Paper IV does not establish an autism mechanism or an APOE-autism causal claim. Paper III remains mechanistically independent because apolipoprotein routing does not establish tryptophan/kynurenine flux.

Paper IV is therefore a host-state and load-control paper, not a replacement for Papers I-II.

23. Hard Negatives and Publication Boundaries

- Do not claim direct RUBV-ApoE or RUBV-ApoA-I binding.
- Do not infer ApoE, ApoA-I, ApoB, or any named apolipoprotein from historical LDL/VLDL/HDL density fractions without composition data.
- Do not claim isolated cholesteryl ester is a proven RUBV inhibitor; the recovered simple cholesteryl-oleate dispersion was negative under the historical assay conditions.
- Do not convert historical pH 6.0-6.2 assay buffers into intracellular CPV or lysosomal pH.
- Do not claim APOE genotype causes autism or is a demonstrated RUBV susceptibility factor.
- Do not convert baseline APOE isoform lysosomal phenotypes into RUBV phenotypes without an explicit virus-by-host-state interaction.
- Do not infer ancestry-based lysosomal pH or trafficking biology from population prevalence data.
- Do not convert DENV, WNV, SARS-CoV-2, or any other comparator-virus mechanism into RUBV proof.
- Do not infer treatment efficacy from ApoE/ApoA, particle-state, or cholesterol-flux findings.
- Do not use serum concentration alone as the mechanistic endpoint when the hypothesis is intracellular routing and availability.

24. Evidence Ledger

Class	Highest-value evidence
DIRECT RUBV	Historical LDL/VLDL/HDL particle-state effects; quantitative native/lipid/apo/sonication pattern; CPV/endolysosomal biology; cargo internalization defects; P150N-ARL8A; CH25H/LIPA/CLN8/CTSS human transcript remodeling; human astrocyte/microglial tropism.
DIRECT DENV	ApoA-I/SR-BI attachment; ApoE/VLDL/capsid and NS1-lipoprotein complexes; increased LDL/cholesterol ingress; late-endosomal/lysosomal cholesterol dependence; NPC2 compartment changes.
DIRECT HOST	ApoE/remnant routing; ApoA-I-assisted ApoE recycling; 25HC->ABCA1/ApoE/LDLR control; APOE isoform lysosomal/cholesterol/pH phenotypes; LAL-NPC2/NPC1 flux biology.
MISSING RUBV NAIL	Named Apo interaction; infection-induced ApoE secretion/lipidation/recycling; RUBV x APOE/particle interaction; Apo-dependent change in NPC2 transfer, lysosomal FC residence, or ER arrival; rescue.

25. Conclusion

Paper IV now defines a specific upstream host-state layer rather than a vague apolipoprotein association. Historical RUBV experiments directly establish that structured human lipoprotein particles, their lipid/protein organization, and their physical state materially change the RUBV-facing phenotype. They do not identify ApoE or ApoA-I as the determinant.

Independent host biology then supplies a mechanistic routing fork: ApoE-containing particles can be sorted between recycling/resecretion and lysosomal degradation, while ApoA-I/HDL can stimulate ApoE recycling and cholesterol efflux. RUBV directly induces CH25H, and independent glial 25HC biology can shift ABCA1, ApoE particle output, LDLR-dependent reuptake, and cholesteryl-ester storage. DENV demonstrates at molecular resolution that ApoA-I and ApoE can become direct viral/lipoprotein nodes.

The strongest current Paper IV prediction is therefore an interaction: a defined ApoE/ApoA particle or APOE host state changes the amount and route of lipid cargo entering a RUBV-remodeled cell, which in turn changes the pressure placed on the downstream NPC2-centered cholesterol-availability system.

The evidence reaches the edge of proof only when that interaction is demonstrated experimentally. A virus-by-host-state effect that changes lysosomal commitment, NPC2 availability or transfer, free-cholesterol residence, or ER arrival - and is reversible by targeted perturbation - would strongly establish a RUBV host-modifier mechanism. Baseline Apo differences without a RUBV interaction would not.

Paper IV is therefore falsifiable at its center: if defined Apo/particle states do not modify RUBV-associated intracellular routing or cholesterol throughput despite robust infection and sensitive stage-resolved measurements, the central host-state hypothesis should be revised or rejected.

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SEPTEMBER 27, 2026 SYNCHRONIZATION ADDENDUM

Paper IV remains the upstream host-state and load-control paper. This addendum corrects the downstream bottleneck language to the current Paper I architecture and defines how Papers VI and VII change the experimental context without converting ApoE/ApoA biology into an autism claim or a demonstrated RUBV susceptibility factor.

26. CORRECTION: PAPER IV NOW FEEDS A LAL-CENTERED BOTTLENECK #1

Older Paper IV language places the leading downstream bottleneck at NPC2-mediated post-LAL cholesterol availability. The current Jacob model supersedes that assignment. Bottleneck #1 is LAL-centered lysosomal cholesteryl-ester/triglyceride throughput and capacity, with the proposed slow handoff after LAL hydrolysis and before NPC2 receives free cholesterol. NPC2 remains essential downstream transfer machinery and a competing discriminator.

Paper IV's core role is unchanged: ApoE/ApoA-defined particle composition, receptor choice, recycling, and lysosomal commitment may change the load delivered to the infected endolysosomal system. The updated prediction is that this input state changes pressure on LAL-centered CE/TG throughput and the post-LAL/pre-NPC2 handoff, not on an assumed NPC2 bottleneck alone.

A particle state that increases lysosomal commitment could increase CE/TG processing demand or expose a slow post-hydrolysis transfer step. A state favoring recycling or efflux could reduce that load. These remain flux predictions. Direct RUBV measurement of particle-derived CE delivery, LAL hydrolysis, free-cholesterol residence, NPC2 receipt/transfer, and ER arrival is required to locate the limiting step.

27. UPDATED FOUR-GATE INTERPRETATION

Gate 1 remains lipoprotein input and recycling versus lysosomal commitment. Gate 2 remains soluble lysosomal cargo delivery and trafficking. Gate 3 remains deep lysosomal chemistry, including pH and SM/ceramide/BMP-related transfer competence. Gate 4 remains downstream sterol redistribution and ER arrival. The correction is kinetic: the lead candidate bottleneck is now LAL-centered throughput with a proposed post-LAL/pre-NPC2 slowdown, while NPC2/NPC1 and post-NPC1 routes remain downstream discriminators that must be measured rather than presumed defective.

28. PAPER VI SYNCHRONIZATION: HOST STATE IS NOT DISEASE CAUSATION

Paper VI extends the research program to a causal hypothesis for a biologically defined autism subtype, particularly developmental regression, and imposes direct mechanism, human-phenotype, MMR/vaccine-strain, and temporal tests. Paper IV does not independently satisfy any of those disease-level requirements.

APOE genotype, ApoE/ApoA particle state, lipoprotein concentration, or a RUBV x host-state interaction must not be treated as an autism marker without a separate prospective disease-level demonstration. If a Paper IV modifier is eventually established, it can be tested as one susceptibility or severity variable inside the Paper VI design; it cannot be promoted to a causal autism factor from cellular routing data alone.

29. PAPER VII SYNCHRONIZATION: UPSTREAM LOAD MAY REACH THE MITOCHONDRIAL BRANCH

Paper VII adds Bottleneck #2 at the mitochondrial outer membrane: MAO. Direct RUBV evidence establishes mitochondrial engagement and functional perturbation, while independent host experiments establish that MAO activity is sensitive to membrane lipid context. Host sterol biology further shows that cholesterol-rich lysosomes can expand STARD3-dependent contacts with mitochondria in NPC1-deficient or inhibited systems.

This creates a new downstream question for Paper IV: can an ApoE/ApoA-defined input state change not only lysosomal CE/TG throughput and cholesterol residence, but also lysosome-mitochondria contact or mitochondrial outer-membrane lipid exposure during RUBV infection? That possibility is experimentally legitimate but not demonstrated.

Paper IV therefore gains a second downstream readout without changing its upstream identity. The primary interaction remains RUBV x particle/APOE state. If that interaction alters LAL/post-LAL flux, STARD3-dependent contact frequency, mitochondrial membrane cholesterol/phospholipids, and MAO-A or MAO-B function in a linked and rescuable sequence, the host-state model would extend across both proposed bottlenecks. If mitochondrial/MAO endpoints remain normal, Paper IV can still be valid as an endolysosomal load-control mechanism.

30. UPDATED DECISIVE EXPERIMENT

Retain the isogenic APOE2/APOE3/APOE4 human glial design and chemically defined ApoA-I/HDL and ApoE/remnant-particle conditions. Quantify particle composition, binding, uptake, recycling, lysosomal commitment, RUBV burden/stage, CH25H/25HC, ApoE secretion/lipidation, ApoA-I recycling, LRP1, LDLR, SR-BI, and ABCA1.

Trace particle-derived CE through lysosomal delivery, direct LAL hydrolysis, free-cholesterol generation and residence, NPC2 receipt/transfer, NPC1-dependent export, ER arrival, and SOAT re-esterification. Add STARD3 localization/contact frequency and mitochondrial outer-membrane cholesterol/phospholipid measurements. MAO-A and MAO-B activity may be measured as downstream Paper VII endpoints, but they are not required to validate Paper IV's upstream modifier hypothesis.

The central statistical test remains an interaction term: particle/APOE state x RUBV. Baseline Apo differences without an infection interaction do not validate Paper IV. A positive interaction should then be localized by flux and rescue rather than assigned automatically to LAL, NPC2, mitochondria, or MAO.

31. UPDATED PUBLICATION BOUNDARY

Do not describe Paper IV as feeding an established NPC2 bottleneck; the current lead is LAL-centered throughput with a proposed post-LAL/pre-NPC2 handoff. Do not claim direct RUBV inhibition of LAL or NPC2. Do not infer a mitochondrial or MAO effect from Apo/particle state without direct measurement. Do not convert APOE genotype or lipoprotein state into an autism susceptibility claim without a separate disease-level interaction. Comparator-virus evidence remains comparator evidence.

32. UPDATED CONCLUSION

Paper IV remains a falsifiable host-state model: structured lipoprotein input can alter receptor routing, recycling, lysosomal commitment, and therefore the lipid load presented to a RUBV-remodeled cell. The current downstream target is broader and more precise than the September 11 formulation: LAL-centered CE/TG throughput, the post-LAL/pre-NPC2 handoff, downstream cholesterol availability, and, as a new Paper VII extension, possible sterol-responsive lysosome-mitochondria communication.

The decisive result remains a RUBV x particle/APOE-state interaction that changes intracellular lipid routing or flux and can be localized and rescued. Without that interaction, ApoE/ApoA biology remains relevant host physiology rather than an established RUBV modifier.

ADDITIONAL CROSS-PAPER REFERENCES

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