

JACOB'S MODEL - PAPER III

RUBELLA VIRUS AND TRYPTOPHAN ROUTING

Direct RUBV Evidence for Interferon/Kynurenine-Pathway Engagement
and a Decisive Human-Neural Flux Test

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Jacob's Voice

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

Scope

This paper examines whether Rubella virus (RUBV) redirects tryptophan through the kynurenine pathway in human neural systems. It is an independent metabolic and neuroimmune branch of Jacob Boatman's broader Rubella research program. It does not require the lysosomal cholesterol model in Paper I, and it does not claim that pathway engagement by itself establishes the autism mechanism considered in Paper II.

Serotonin is not a causal component of the current Jake model. This paper does not use serotonin depletion, serotonin excess, serotonin-receptor competition, or altered 5-HT signaling as a required bridge.

Author's Note - Origin and Governance of the Model

The routing hypothesis presented here originated with Jacob Boatman. Jacob repeatedly framed the biological problem as one of movement, allocation, and use rather than simple abundance. In the tryptophan branch, the resulting question is whether RUBV redirects a substrate pool into a different metabolic route and whether that change can be measured in the cell types most relevant to the developing human brain.

Scott Boatman serves as coauthor, literature investigator, and translator of Jacob's mechanistic descriptions into experimentally testable questions. The evidence determines the strength of each arrow. Direct RUBV observations, direct host capacity, adjacent context, and missing same-system measurements remain explicitly separated.

Abstract

Paper III now rests on an unusually compact evidence staircase. In primary human endothelial cells, RUBV produces extremely large direct transcriptional engagement of the kynurenine pathway. In the Geyer dataset, IDO1 rises approximately 644.9-fold in fetal endothelial cells and 286.3-fold in adult endothelial cells. KYNU is induced across multiple probes, including approximately 203.5-fold in fetal cells and approximately 75.0-, 54.6-, 53.6-, and 33.3-fold in adult cells. KMO rises approximately 23-fold in adult endothelial cells. These are direct RUBV-associated transcript measurements, not inferred pathway activity.

Independent evidence strengthens each side of that signal. Bilz and colleagues validated a positive increase in KYNU mRNA across tested RUBV isolates and placed the response beside MX1/MX2 interferon-stimulated genes and SOD2 redox/mitochondrial remodeling. In developing human brain tissue and microglia-containing organoids, RUBV predominantly infects microglia and produces a profound interferon response in neighboring neurons and neural progenitor cells. Independently, stable-isotope experiments show that human microglia can convert labeled L-tryptophan through kynurenine to labeled quinolinic acid, and interferon-gamma markedly increases kynurenine accumulation.

Taken together, the evidence directly establishes three components required by the model: RUBV can create the relevant interferon-rich developing-human-brain environment; RUBV can strongly engage IDO1/KYNU/KMO-associated transcription in primary human cells; and human microglia possess the exact tryptophan-to-kynurenine-to-quinolinate flux capacity proposed downstream. The remaining gap is specific rather than vague: no recovered RUBV experiment directly measures isotope-resolved tryptophan flux through this pathway in a human microglia-containing neural system.

We therefore propose a decisive same-system experiment using stable-isotope-labeled tryptophan, cell-type-resolved metabolite measurements, and independent IFNAR and IDO1 perturbation. A reproducible increase in labeled tryptophan-to-kynurenine flux that is reduced by IFNAR or IDO1 perturbation would establish an interferon/IDO1-dependent RUBV metabolic routing phenotype. A well-powered negative result despite robust infection, interferon response, pathway transcription, and assay sensitivity would substantially falsify the central Paper III hypothesis.

1. What Is Already Established - and What Is Not

Paper III begins beyond plausibility. RUBV has already been shown to engage the molecular machinery of the kynurenine pathway in primary human cells at very large magnitude. The unresolved issue is metabolic throughput: whether those transcriptional changes cause labeled tryptophan carbon to move into kynurenine and downstream metabolites during RUBV infection.

Evidence level	Observation	Status
DIRECT RUBV	IDO1 rises ~644.9-fold in fetal endothelial cells and ~286.3-fold in adult endothelial cells.	Established transcriptional engagement at pathway entry.
DIRECT RUBV	KYNU rises across multiple probes, including ~203.5-fold fetal and ~75.0, 54.6, 53.6, and 33.3-fold adult.	Established downstream-pathway transcriptional engagement.
DIRECT RUBV	KMO rises ~23-fold in adult endothelial cells.	Established engagement of the 3-hydroxykynurenine branch at transcript level.
DIRECT RUBV	Bilz independently validates positive KYNU mRNA direction across tested RUBV isolates, alongside MX1/MX2 and SOD2 response.	Independent directionality support.
DIRECT HUMAN NEURAL RUBV	RUBV predominantly infects microglia in developing-human-brain models and drives profound interferon responses in neighboring neurons/progenitors.	Established relevant neural/interferon context.
DIRECT HUMAN HOST FLUX	Human microglia convert isotope-labeled tryptophan through kynurenine to labeled quinolinic acid; IFN-gamma increases kynurenine.	Established metabolic capacity of the target host cell.
NOT YET SHOWN	RUBV increases isotope-resolved TRP->KYN->downstream flux in human microglia-containing neural tissue.	The decisive missing experiment.

The paper therefore makes a narrow strong claim: RUBV engagement of IDO1/KYNU/KMO-associated machinery is direct; the conversion of that engagement into altered metabolic flux remains to be demonstrated.

2. Central Question

The central question is no longer whether RUBV can engage the molecular neighborhood of the kynurenine pathway. It can. The unresolved question is whether that engagement changes metabolic throughput in human neural cells.

Specifically: does RUBV merely induce IDO1, KYNU, and KMO-associated transcription, or does it measurably redirect tryptophan carbon into kynurenine and downstream metabolites? Transcript abundance and metabolic flux are not interchangeable. Paper III is designed around that distinction.

3. The Host Tryptophan-to-Kynurenine Route

L-tryptophan -> IDO1/TDO-dependent pathway entry -> L-kynurenine -> branching toward kynurenic acid or 3-hydroxykynurenine -> KYNU and downstream reactions -> 3-hydroxyanthranilic acid/quinolinic acid -> downstream NAD-related metabolism.

This pathway is branched rather than linear. IDO1 can control an important inflammatory entry step, but downstream metabolite balance depends on cell type, enzyme expression, cofactors, substrate availability, redox state, and competing branch enzymes. A strong transcriptional signature therefore cannot by itself establish whether the dominant metabolic output is kynurenine, kynurenate, 3-hydroxykynurenine, quinolinate, or increased de novo NAD-related flux.

5. Quantitative Magnitude of the RUBV Kynurenine-Pathway Signal

The magnitude of the Geyer signal deserves interpretation beyond a gene list. IDO1 induction is measured in hundreds-fold in both fetal and adult primary endothelial cells. KYNU is represented by several independent

strongly positive probes rather than a single isolated feature. Adult-cell KMO induction extends the signal into a downstream branch that can feed 3-hydroxykynurenine chemistry.

Gene/probe pattern	Fetal primary endothelial cells	Adult primary endothelial cells	Strict interpretation
IDO1	~644.9-fold; $p=3.68 \times 10^{-4}$	~286.3-fold; $p=7.45 \times 10^{-4}$	Massive direct RUBV-associated transcript induction.
KYNU	~203.5-fold; additional ~37.6- and ~6.5-fold probes	~75.0, 54.6, 53.6, and 33.3-fold probes	Multi-probe downstream pathway engagement.
KMO	No qualifying filtered hit preserved	~23.2 and ~23.0-fold	Adult-cell 3-HK-branch engagement.
QPRT/HAAO/NADSYN1	No qualifying filtered-table call recovered	No qualifying filtered-table call recovered	No claim of downstream NAD-pathway induction from this dataset.

These values establish transcriptional engagement, not enzyme activity. They do not show tryptophan depletion, increased KYN/TRP ratio, 3-hydroxykynurenine production, quinolinate production, or de novo NAD synthesis. The strength of Paper III comes from keeping that boundary intact while recognizing that the upstream RUBV signal itself is unusually large.

5. Direct RUBV Evidence: IDO1, KYNU, and KMO

Geyer and colleagues profiled RUBV-infected primary endothelial cells of fetal and adult origin. The filtered supplementary dataset contains one of the strongest direct metabolic-transcription signals in the current Jacob program.

Target	Fetal endothelial cells	Adult endothelial cells	Evidence interpretation
IDO1	~644.9-fold; $p=3.68 \times 10^{-4}$	~286.3-fold; $p=7.45 \times 10^{-4}$	Very large direct RUBV-associated transcript induction.
KYNU	Multiple probes including ~203.5-, 37.6-, and 6.5-fold	Multiple probes including ~75.0-, 54.6-, 53.6-, and 33.3-fold	Direct multi-probe engagement of kynureninase transcription.
KMO	No qualifying hit preserved in the filtered list	~23.2- and 23.0-fold	Direct adult-endothelial engagement of the 3-HK branch.

The magnitude matters. IDO1 is not a marginal signal: the filtered analysis shows several-hundred-fold induction in both fetal and adult primary endothelial cells. KYNU is independently represented by multiple strongly positive probes, and KMO is strongly induced in adult cells. These findings establish direct RUBV transcriptional engagement across pathway entry and downstream branch machinery.

The boundary is equally important. These are microarray transcript measurements. They do not establish protein abundance, enzyme activity, tryptophan disappearance, KYN/TRP ratio, or metabolite flux. No QPRT, HAAO, or NADSYN1 entry meeting the filtered-table threshold was recovered in the current review; absence from a filtered list is not evidence of no biological change.

6. Independent RUBV Validation: KYNU Directionality Is Positive

Bilz and colleagues independently studied RUBV-associated metabolic remodeling and validated selected differentially expressed genes by RT-qPCR across distinct RUBV isolates. KYNU was explicitly included among the remarkable infection-associated changes, and the authors reported a positive influence of RUBV on KYNU mRNA expression.

This independent validation matters because it moves the RUBV-to-KYNU relationship beyond a single microarray dataset. The correct statement is not merely that RUBV 'alters' KYNU. In the tested systems, RUBV increased KYNU mRNA.

The same validated response set included MX1 and MX2, canonical interferon-stimulated genes, and SOD2, a mitochondrial antioxidant enzyme. Together with the broader Bilz metabolic phenotype, these findings

place KYNU induction inside an interferon-rich, redox- and bioenergetically remodeled RUBV state. They do not show that MX1, MX2, or SOD2 causes the kynurenine phenotype.

7. Why Interferon Is the Mechanistic Bridge, Not a Background Marker

The interferon connection is not introduced solely from general immunology. In RUBV systems, MX1/MX2 validate an interferon-rich transcriptional state, and the developing-human-brain work shows a profound interferon response in neighboring neural cells during microglial RUBV infection. Independently, interferon-gamma increases kynurenine accumulation in human microglia.

These observations define a testable bridge: RUBV-associated interferon signaling may be upstream of IDO1-dependent metabolic routing. They do not prove that type I/III interferon in the RUBV neural model is identical to IFN-gamma stimulation in the historical microglial flux experiment. That is why IFNAR and IDO1 perturbations are both required in the decisive experiment.

A positive IFNAR perturbation result would connect the RUBV interferon environment to the metabolic phenotype. A positive IDO1 perturbation result would establish pathway-entry dependence. If flux persists despite both perturbations, the model must be revised toward another pathway-entry mechanism.

8. Developing Human Brain: RUBV, Microglia, and Interferon

Popova and colleagues used human fetal brain slices and microglia-containing organoids. RUBV predominantly infected microglia and produced a profound interferon response in non-microglial cells, including neurons and neural progenitor cells. The presence of microglia altered the magnitude of that response.

This is a critical direct bridge for Paper III. The metabolic hypothesis is not being imported into an unrelated tissue after the fact. RUBV directly enters a developing-human-brain system containing microglia, while neighboring neural cells mount strong interferon responses. The study did not measure tryptophan metabolites, so it establishes the neural interferon context rather than pathway flux.

9. Human Microglia Can Convert Tryptophan to Quinolinic Acid

Heyes and colleagues incubated human microglia, macrophages, and human fetal brain-cell cultures with stable-isotope-labeled L-tryptophan. Human microglia produced labeled kynurenine and labeled quinolinic acid. Interferon-gamma substantially increased kynurenine accumulation, and microglia produced far more labeled quinolinic acid than mixed human fetal brain cultures.

This is direct functional host evidence. Human microglia possess the exact isotope-resolved tryptophan-to-kynurenine-to-quinolinate capacity required by the proposed downstream route. It is not a RUBV experiment. The missing experiment is to place the RUBV neural system and this flux measurement in the same system.

10. The Human-Microglia Flux Experiment Defines the Required Endpoint

The Heyes experiment is important because it measures what the RUBV transcript studies do not: movement of labeled substrate through the pathway. Human microglia exposed to stable-isotope-labeled L-tryptophan generated labeled kynurenine and labeled quinolinic acid. This demonstrates actual carbon transfer through the pathway rather than inferred pathway activation.

The same experiment also establishes cell-type relevance. Human microglia produced substantially more labeled quinolinic acid than mixed human fetal brain-cell cultures, and IFN-gamma increased kynurenine accumulation. Thus microglia are not merely a convenient cell type for the proposed experiment; they are a directly demonstrated human neural-immune cell capable of the downstream flux being tested.

The missing experiment is therefore conceptually simple: repeat the logic of isotope tracing in the RUBV neural system that directly shows microglial infection and neighboring interferon responses.

11. The Evidence Staircase Is Now Narrow and Strong

Paper III no longer depends on a long chain of weak analogies. Three major components are directly established in separate human systems: RUBV produces a profound interferon environment in developing human neural tissue; RUBV strongly induces IDO1/KYNU/KMO-associated transcription in primary human cells; and human microglia directly convert labeled tryptophan through kynurenine to labeled quinolinic acid.

The fourth component - RUBV-driven metabolite flux in the same microglia-containing neural system - remains unmeasured. That is the decisive gap. The hypothesis is therefore both strong and unusually falsifiable: one experiment can connect the existing evidence staircase or break it.

12. Current Jacob Tryptophan-Routing Model

RUBV infection in a susceptible neural context -> strong interferon response -> IDO1 transcriptional engagement -> increased routing of tryptophan into kynurenine? -> KYNU/KMO branch engagement -> altered downstream kynurenine-metabolite balance? -> altered neuroimmune/redox/NAD-related state?

The question marks are deliberate. RUBV infection, the interferon-rich neural context, and pathway transcription have direct support. Increased metabolic flow and downstream metabolite direction have not been demonstrated during RUBV infection.

Serotonin is not required by this model. The pathway can influence neural biology through kynurenine metabolites, immune-metabolic state, oxidative chemistry, and NAD-related metabolism without invoking serotonin depletion, excess, or receptor competition.

13. Metabolite Direction Cannot Be Assumed from Gene Direction

Even if RUBV increases tryptophan entry into the kynurenine pathway, the downstream biological result remains branch-dependent. Kynurenine can be routed toward kynurenic acid or toward KMO-dependent 3-hydroxykynurenine chemistry and subsequent metabolites. KYNU and KMO transcript induction makes the latter branch experimentally important, but it does not establish its dominance.

For that reason, Paper III does not use a single KYN/TRP ratio as the final endpoint. A decisive profile must quantify labeled tryptophan disappearance and labeled appearance in kynurenine, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, and downstream NAD-related pools where technically feasible.

A rise in total quinolinate without labeled contribution from the supplied tryptophan would not prove increased pathway flux from that substrate. Isotopologue resolution is what separates pool size from routing.

14. Timing, Persistence, and State Dependence

The decisive experiment should not treat the RUBV response as a single static time point. Interferon induction, transcriptional engagement, metabolic adaptation, and persistent infection can occupy different phases. The same pathway may therefore behave differently during early antiviral signaling, established infection, and persistent states.

Wild-type clinical RUBV can establish persistent productive infection in primary human fetal endothelial cells with little cytopathology and continued infectious-virus release through serial passage. That persistence evidence does not demonstrate chronic kynurenine-pathway flux, but it makes duration a legitimate experimental variable rather than an assumption.

The experimental design should therefore span early interferon induction through established infection and, where technically feasible, persistent-state conditions. Acute interferon-linked flux must be distinguished from a metabolic phenotype that persists after the initial interferon peak.

15. Stage-Resolved Prediction Matrix

Stage	Primary measurements	Interpretive purpose
Early infection / interferon rise	Viral burden, IFN program, IDO1	Determine whether pathway entry begins with

Stage	Primary measurements	Interpretive purpose
	transcript/protein, labeled TRP uptake	antiviral signaling.
Established infection	TRP, KYN, KYN/TRP, isotope-resolved KYN and branch metabolites, IDO1/KYNU/KMO protein/activity	Determine whether transcription becomes metabolic throughput.
Late / adapted state	3-HK, KYNA, 3-HAA, QUIN, NAD-related labeling, redox/SOD2 state	Determine branch direction and downstream persistence.
Persistent infection where feasible	Same flux panel plus viral production and interferon state	Separate sustained routing from transient acute response.

A single early endpoint is insufficient. Conversely, a late metabolite shift cannot be assumed to have been present during entry. The pathway must be followed through time.

16. Cell-Type Resolution Is Required

Whole-culture metabolomics is not sufficient for the central claim. RUBV can preferentially infect microglia while neighboring neurons and progenitors show strong non-cell-autonomous interferon responses. Conversely, the largest direct IDO1/KYNU/KMO transcript magnitudes were measured in endothelial cells.

A whole-organoid increase in kynurenine would establish system-level rerouting but would not identify the producing cell. The strongest experiment should determine, as far as technically feasible, which cell population consumes labeled tryptophan, produces labeled kynurenine, and contributes labeled carbon to downstream metabolites.

17. Relationship to Papers I and II

Paper III is independent of the lysosomal cholesterol mechanism in Paper I. NPC2, LAL, CI-M6PR, lysosomal pH, SGMS1, BMP/LBPA, lipoprotein architecture, and post-NPC1 cholesterol transfer are not evidence that RUBV redirects tryptophan.

Paper III sits most naturally beside the glial/interferon route in Paper II. If RUBV activates interferon programs in developing neural systems and simultaneously drives measurable IDO1-dependent tryptophan flux, the kynurenine pathway becomes a concrete metabolic output of that host response.

A positive Paper III result would not, by itself, prove an autism mechanism. It would establish a RUBV-associated neuroimmune metabolic mechanism that could then be tested separately against neurodevelopmental endpoints.

18. Direct, Adjacent, and Missing Evidence

Status	Finding	Boundary
DIRECT RUBV	IDO1 ~644.9-fold fetal and ~286.3-fold adult; large multi-probe KYNU induction; adult KMO ~23-fold in Geyer primary endothelial cells.	Transcript abundance, not metabolic flux.
DIRECT RUBV	Bilz validates positive KYNU mRNA direction across tested RUBV isolates; MX1/MX2 and SOD2 co-validate interferon/redox context.	No kynurenine-pathway metabolites measured.
DIRECT RUBV / HUMAN NEURAL	Developing human brain models show predominant microglial infection and profound interferon responses in surrounding neurons/progenitors.	No tryptophan tracing.
DIRECT HOST	Human microglia convert labeled tryptophan to labeled kynurenine and quinolinic acid; IFN-gamma increases kynurenine.	Not performed with RUBV.
MISSING SAME-SYSTEM RUBV	TRP disappearance, KYN increase, KYN/TRP ratio, 3-HK, KYNA, 3-HAA, QUIN, or labeled TRP contribution to	This is the decisive gap.

Status	Finding	Boundary
	NAD-related metabolites in RUBV-exposed human neural systems.	

19. What Would Convert Paper III from Convergence to Direct RUBV Metabolic Mechanism

The evidence crosses from convergence to direct mechanism if the same human-relevant RUBV system shows an increase in isotope-resolved tryptophan-to-kynurenine flux and that increase is reduced by pathway-specific perturbation.

The strongest result would contain four linked observations: robust RUBV infection with the expected interferon response; increased labeled TRP->KYN flux; branch-resolved labeled downstream metabolites; and loss or rescue of the flux phenotype after IFNAR, IDO1, and where indicated KMO/KYNU perturbation.

A transcript-only result, even one reproducing the hundreds-fold IDO1 signal, does not meet this threshold. A metabolite pool change without isotope tracing does not fully meet it either. The central claim is routing, so the decisive evidence must measure routing.

20. Decisive Experiment: Stable-Isotope Tryptophan Flux During RUBV Infection

13.1 Model system

- Human microglia-containing neural organoids or a validated microglia-neuron/progenitor coculture.
- Matched microglia-free neural controls where feasible.
- Matched mock and active RUBV conditions, with viral burden quantified by cell type.
- A stage-aware time course spanning early interferon induction, established infection, and persistent-state conditions where feasible.

13.2 Flux tracer and required metabolites

- Introduce stable-isotope-labeled tryptophan and quantify isotopologue disappearance and appearance rather than inferring flux from transcripts.
- Measure tryptophan, kynurenine, KYN/TRP ratio, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, and labeled contribution to downstream NAD-related metabolites where technically feasible.
- Quantify extracellular and intracellular pools so increased production can be distinguished from altered export or uptake.

13.3 Pathway and causal measurements

- Measure IDO1, KYNU, KMO, HAAO, QPRT, and relevant branch enzymes at transcript and protein levels; directly measure IDO1 activity where feasible.
- Resolve interferon signaling by cell type and include MX1/MX2 as response anchors rather than causal surrogates.
- Measure SOD2, cellular redox state, and NAD⁺/NADH when testing the downstream redox/NAD branch.
- Perturb IFNAR and IDO1 independently. If labeled 3-HK or QUIN changes, perturb KMO/KYNU to map the responsible downstream branch.
- Require rescue or loss of isotope-resolved flux for causal interpretation. Transcript normalization alone is insufficient.

21. Predetermined Interpretation Matrix

Observed result	Interpretation
IDO1/KYNU/KMO transcripts rise, but isotope-resolved	Transcriptional engagement without meaningful rerouting;

Observed result	Interpretation
TRP/KYN flux is unchanged.	central flux hypothesis substantially weakened.
Labeled TRP->KYN flux rises and falls with IFNAR or IDO1 perturbation.	Strong support for a RUBV-associated interferon/IDO1-dependent routing phenotype.
KYN flux rises but downstream labeled metabolites do not.	Pathway entry changes; the downstream branch remains unproven.
Labeled 3-HK/QUIN rises in microglia-containing systems and falls with KMO/KYNU perturbation.	Support for a microglial downstream kynurenine branch.
Flux change occurs only in endothelial/non-neural systems.	Limits neurodevelopmental relevance of Paper III.
No reproducible isotope-flux effect despite robust infection, interferon response, pathway transcription, tracer recovery, and assay sensitivity.	Central Paper III routing hypothesis substantially falsified.

22. Alternative Explanations

- IDO1/KYNU/KMO induction may be part of a broad antiviral transcriptional program without material change in substrate flux.
- Endothelial transcriptional responses may not reproduce the magnitude or direction of metabolism in microglia, neurons, or progenitors.
- RUBV-induced interferon may increase pathway entry while cofactor or downstream-enzyme limitations prevent quinolinate or NAD-related changes.
- Different cell types may route kynurenine into different branches, making bulk metabolomics misleading.
- Any observed kynurenine shift could reflect generalized inflammatory stress rather than a RUBV-specific mechanism.
- Strain, developmental stage, multiplicity of infection, persistence state, nutrient availability, and microglial abundance may materially change the phenotype.

23. Falsification Criteria

Paper III should be considered substantially falsified in its current form if well-powered human-relevant RUBV experiments show robust infection, a strong interferon response, and reproducible IDO1/KYNU/KMO transcriptional engagement but no increase in isotope-resolved tryptophan-to-kynurenine flux, no consistent change in KYN/TRP ratio, and no downstream labeled-metabolite change.

A negative result is especially strong if tracer recovery, assay sensitivity, cell-type representation, infection burden, and time-window selection are validated. The model must not be rescued by substituting transcript abundance, inflammatory markers, or comparator-virus biology for missing flux.

24. Publication Boundaries

- Do not claim RUBV has been shown to deplete tryptophan in human neural tissue.
- Do not claim RUBV has been shown to increase kynurenine, 3-HK, kynurenate, quinolinate, or NAD-related metabolites until they are directly measured during RUBV infection.
- Do not convert IDO1/KYNU/KMO transcript induction into enzyme activity or pathway flux.
- Do not assign endothelial transcript magnitudes to microglia, neurons, or neural progenitors.
- Do not infer quinolinic-acid production from KYNU/KMO induction alone.
- Do not insert serotonin as a causal bridge.
- Do not insert NPC2, LAL, APOE/APOA, lysosomal pH, SGMS1, BMP/LBPA, or other cross-paper findings as causal Paper III arrows without direct metabolic evidence.
- Do not infer autism causation from a positive kynurenine-flux phenotype alone.

- Do not infer persistent RUBV from Rubella antibody titers.
- Do not rescue a negative isotope experiment with transcript abundance or inflammatory-marker changes.

25. Why Paper III Is the Cleanest Direct Metabolic Branch

Paper III has one of the strongest direct evidence-to-gap ratios in the program. RUBV directly creates the relevant interferon-rich developing-human-brain environment. RUBV directly drives very large IDO1 and KYNU transcriptional responses and measurable KMO induction in primary human cells. KYNU directionality is independently validated across tested RUBV isolates. Human microglia independently possess the precise stable-isotope tryptophan-to-kynurenine-to-quinolinate capacity required by the proposed route.

The missing bridge is singular: metabolic throughput during RUBV infection in that human neural context. That makes the hypothesis unusually testable. A positive isotope-resolved result would convert a strong transcriptional and cellular convergence into direct metabolic mechanism. A clean negative result would require the routing hypothesis to be revised or rejected.

Evidence Ledger: What a Reader Can Reuse

Class	Reusable result
DIRECT RUBV	Primary human-cell IDO1/KYNU/KMO transcriptional engagement with exact fold-change anchors.
DIRECT RUBV	Independent positive KYNU directionality across tested isolates with MX1/MX2 and SOD2 context.
DIRECT HUMAN NEURAL RUBV	Microglial tropism plus profound neighboring neural-cell interferon responses in developing-human-brain models.
DIRECT HUMAN HOST FLUX	Stable-isotope TRP->KYN->QUIN conversion by human microglia; IFN-gamma increases KYN accumulation.
HARD BOUNDARY	No recovered same-system RUBV human-neural isotope-flux experiment.
DECISIVE NEXT STEP	RUBV + labeled TRP + cell-type-resolved metabolites + IFNAR/IDO1 perturbation, with KMO/KYNU branch tests where indicated.

This ledger is intentionally compact. Paper III should remain useful even to a reader who rejects the proposed RUBV routing mechanism: it assembles the direct RUBV transcriptional evidence, the developing-human-brain interferon evidence, the direct human-microglia flux capacity, and the exact missing experiment in one place.

Conclusion

The strongest current statement is straightforward. RUBV directly engages the kynurenine pathway at the transcriptional level with unusually large magnitude: IDO1 rises by hundreds-fold in fetal and adult primary endothelial cells, KYNU rises strongly across multiple probes and is independently validated as increased across tested RUBV isolates, and KMO is strongly induced in adult endothelial cells.

RUBV also directly creates the relevant developing-human-brain context. It predominantly infects microglia and produces profound interferon responses in neighboring neurons and neural progenitor cells. Independently, human microglia can convert labeled tryptophan through kynurenine to labeled quinolinic acid, and interferon-gamma increases kynurenine accumulation.

Those findings do not yet prove RUBV-driven tryptophan rerouting. They make the missing experiment precise. The decisive question is whether RUBV infection changes where labeled tryptophan carbon goes in a human microglia-containing neural system, and whether that change depends on interferon/IDO1 signaling.

If RUBV increases isotope-resolved TRP->KYN flux and that flux is reduced by IFNAR or IDO1 perturbation, Paper III becomes a direct RUBV metabolic mechanism. If robust RUBV infection and pathway transcription occur without measurable flux change, the central routing hypothesis should be rejected or substantially revised.

The core proposition is not that tryptophan is simply high or low. It is that presence does not equal use. Paper III tests whether Rubella changes the route.

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SEPTEMBER 27, 2026 SYNCHRONIZATION ADDENDUM

Paper III remains an independent RUBV interferon/kynurenine-pathway paper. Papers VI and VII do not replace its central isotope-flux test and do not authorize cross-paper mechanisms to be inserted into the tryptophan route without direct evidence. This addendum defines the allowed connections while preserving Paper III's deliberately narrow metabolic claim.

26. PAPER VI SYNCHRONIZATION: DISEASE-LEVEL BOUNDARY

Paper VI strengthens the broader research program's disease-level test by focusing on a biologically defined autism subtype, particularly developmental regression, and by requiring mechanism, human phenotype, and temporal ordering to converge. A positive Paper III isotope-flux result would supply a RUBV-associated neuroimmune metabolic mechanism; it would not, by itself, establish autism causation, developmental regression, or the endolysosomal cholesterol mechanism.

The correct disease-level sequence is therefore two-stage. First establish RUBV-driven tryptophan routing in a human microglia-containing neural system using isotope-resolved flux and IFNAR/IDO1 perturbation. Only then test whether that metabolic phenotype is enriched, temporally ordered, or functionally relevant in a prospectively defined neurodevelopmental subgroup. Paper III must not use diagnosis, vaccination history, antibody titer, or downstream lipid findings as substitutes for the missing metabolic experiment.

27. PAPER VII SYNCHRONIZATION: MAO IS NOT A SEROTONIN BRIDGE

Paper VII identifies MAO as Bottleneck #2 in Jacob's current two-bottleneck architecture and assembles direct RUBV mitochondrial evidence with independent membrane/lipid-sensitive MAO biology. That finding does not restore serotonin as a causal node in Paper III. Serotonin remains excluded from the current Jacob tryptophan-routing model unless Jacob explicitly revises that decision.

MAO and the kynurenine pathway may occupy the same broader mitochondrial, redox, and neuroimmune landscape, but no recovered evidence establishes a RUBV-specific causal sequence from MAO-A or MAO-B dysfunction to IDO1/KYNU/KMO flux, or from kynurenine flux to MAO dysfunction. The branches therefore remain experimentally independent.

Paper VII does create one legitimate experimental opportunity: the same synchronized RUBV neural system can measure isotope-resolved tryptophan routing and mitochondrial/MAO state in parallel. Parallel measurement can test covariance without presuming causation. If both phenotypes occur, perturbation is still required to determine whether they are linked or merely co-occur during infection.

28. INTEGRATED MULTI-BRANCH EXPERIMENT WITHOUT CROSSING THE EVIDENCE LINE

The highest-information design now uses matched mock and RUBV human microglia-containing neural cultures sampled across early, established, late/adapted, and persistent states where feasible. Preserve Paper III's primary endpoints: labeled tryptophan uptake and disappearance; labeled kynurenine, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid, and NAD-related pools where technically feasible; IDO1/KYNU/KMO abundance and activity; and IFNAR/IDO1 perturbation.

In parallel, Paper VII endpoints may be collected from the same system: MAO-A and MAO-B catalytic activity separately, mitochondrial membrane lipid state, p32 import, respiratory-chain activity, and viral burden. These measurements strengthen discrimination. They do not become Paper III causal arrows unless perturbation demonstrates directionality.

29. PREDETERMINED CROSS-PAPER INTERPRETATION

If RUBV increases isotope-resolved TRP-to-KYN flux while MAO-A and MAO-B remain normal, Paper III is supported independently of Paper VII. If MAO changes while isotope-resolved tryptophan flux remains normal despite strong IDO1/KYNU/KMO transcription, Paper VII can remain viable while Paper III's routing hypothesis weakens. If both change, the result establishes co-occurring RUBV metabolic and mitochondrial phenotypes; it does not establish that one causes the other. If neither changes despite robust infection and validated assays, both functional branches are weakened.

30. UPDATED PUBLICATION BOUNDARY

Do not insert MAO-A, MAO-B, mitochondrial cholesterol, LAL, NPC2, STARD3, MMR, or autism regression as causal steps in the Paper III pathway without direct same-system evidence. Do not infer serotonin depletion, serotonin excess, receptor competition, or 5-HT dysfunction from MAO biology. Do not convert transcript induction into enzyme activity or metabolic flux. Do not convert a positive RUBV kynurenine-flux phenotype into an autism mechanism without a separate disease-level demonstration.

The strongest Paper III statement remains unchanged: RUBV directly engages IDO1/KYNU/KMO-associated transcription and creates a relevant interferon-rich developing-human-brain context; human microglia possess the required tryptophan-to-kynurenine-to-quinolinate flux capacity; the decisive missing experiment is isotope-resolved RUBV-driven flux in that same human neural context.

ADDITIONAL CROSS-PAPER REFERENCES

17. Does Rubella Cause Autism? A Testable Endolysosomal Mechanism Linking RUBV P150-ARL8 Trafficking to Cholesterol Unavailability and Developmental Regression. Jacob's Voice Research Program. Paper VI. September 20, 2026.
18. Jacob's Model - Paper VII. Bottleneck #2: MAO. Jacob Boatman and Scott Boatman. Jacob's Voice. September 27, 2026.
19. Beatch MD, Hobman TC. Rubella virus capsid associates with host cell protein p32 and localizes to mitochondria. J Virol. 2000;74:5569-5576. PMID 10823864.
20. Ilkow CS, Weckbecker D, Cho WJ, et al. The Rubella Virus Capsid Protein Inhibits Mitochondrial Import. J Virol. 2010;84:119-130. PMID 19846524.
21. Claus C, Schonefeld K, Hubner D, et al. Activity increase in respiratory chain complexes by rubella virus with marginal induction of oxidative stress. J Virol. 2013;87. PMID 23720730.