

The REST/STAT3-miR-21 Axis Drives Premature Biological Aging and Mortality in Severe COVID-19 by Silencing p53/PTEN and LKB1-AMPK Checkpoints and Stem Cell Pluripotency

Boominathan Lakshmanane

drboomi@genomediscovery.org

genomediscovery.org <https://orcid.org/0009-0008-5358-7462>

Biological Sciences - Article

Keywords: miR-21-5p, LKB1/STK11, AMPK activation, REST, STAT3, Biological aging, Centenarians, SARS-CoV-2 / Long COVID, Pluripotency factors, Lithocholic Acid.

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11317923/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

The REST/STAT3-miR-21 Axis Drives Premature Biological Aging and Mortality in Severe COVID-19 by Silencing p53/PTEN and LKB1-AMPK Checkpoints and Stem Cell Pluripotency

Dr. L. Boominathan, PhD*

Genome-2-BioMedicine Discovery Center (GBMD), *genomediscovery.org*

Puducherry-605004, India

October 9, 2026

Abstract

Biological aging is characterized by chronic low-grade inflammation, metabolic dysregulation, and progressive loss of stem cell regenerative capacity. However, the upstream triggers that allow severe viral infections, such as SARS-CoV-2, to accelerate biological aging and drive high mortality in younger individuals remain unresolved. Here, using an integrative systems biology framework, non-linear ordinary differential equation (ODE) modeling, and multi-omics analysis, we identify a unified **STAT3-REST-miR-21-5p** regulatory network that acts as an endogenous brake on cellular energy checkpoints, tumor suppression, and pluripotency.

While circulating miR-21-5p, miR-126-3p, miR-146a-5p, and miR-181a-5p progressively increase during typical human aging, ultra-centenarians (100-111 years) maintain suppressed miR-21-5p profiles identical to young adults (18-50 years) (Accardi et al., *Cells*, 2022). We demonstrate that SARS-CoV-2 infection hijacks this centenarian-preserved pathway: induction of RE1-Silencing Transcription Factor (REST) (Atkins et al., *eLife*, 2021) and IL-6/STAT3 signaling drives a pathological surge of miR-21-5p and associated lncRNAs (*NEAT1* and *MALAT1*) in young COVID-19 patients (< 55 years), directly correlating with severe lung/kidney injury, mechanical ventilation (OR = 5.63), and mortality (OR = 6.00) (Bautista-Becerril et al., *Int. J. Mol. Sci.*, 2023).

*Founder, Director-President & Senior Scientist, Genome-2-BioMedicine Discovery Center (GBMD), Puducherry-605004, India. Emails: drboomi@genomediscovery.org, genomdiscovery@gmail.com. Phone: +91-413-2960879.

Mechanistically, elevated miR-21-5p directly targets and silences key tumor suppressors and metabolic controllers, including *TP53* (Gao et al., *Cancer Immunol. Immunother.*, 2023) and *PTEN* (Zhao et al., *Oncol. Rep.*, 2021), while targeting the 3'-UTR of *STK11* (LKB1). This dual-action repression - compounded by the loss of the tumor-suppressive lncRNA *MEG3* - completely blunts Thr172 phosphorylation of AMP-activated protein kinase (AMPK) and overrides anti-aging metabolic signaling, such as the Lithocholic Acid (LCA)-TULP3 axis (Qu et al., *Nature*, 2025). Concurrently, miR-21-5p represses somatic stem cell self-renewal factors (*POU5F1/OCT4*, *NANOG*, *SOX2*, and *MYC*) (Singh et al., *Stem Cells*, 2008), severely impairing tissue regeneration and driving whole-blood transcriptional signatures of age and mortality (Gurinovich et al., *Aging Cell*, 2026). ODE kinetic modeling confirms that this double-negative feedback loop creates a sharp metabolic bifurcation, locking infected young cells into an acute, hyper-aged senescent state. Interruption of this axis via simulated miR-21 antagonists or JAK/STAT3 inhibition restores LKB1-AMPK phosphorylation and unblocks regenerative potential, establishing the REST-miR-21-5p axis as an actionable target to restore healthspan and prevent post-viral mortality.

Keywords: miR-21-5p, LKB1/STK11, AMPK activation, REST, STAT3, Biological aging, Centenarians, SARS-CoV-2 / Long COVID, Pluripotency factors, Lithocholic Acid.

1 Results

1.1 Ultra-Centenarians Avoid the Pro-Aging miR-21-5p Expression Spike

To establish the baseline landscape of microRNA dynamics across human longevity, we evaluated circulating miRNA signatures across age cohorts spanning young adults (18-50 years), middle-aged to octogenarians (51-99 years), and exceptional longevity individuals/ultra-centenarians (100-111 years). While circulating levels of age-associated miRNAs—including miR-21-5p, miR-126-3p, miR-146a-5p, and miR-181a-5p—exhibit a progressive, statistically significant increase during standard biological aging (peaking in the 71-99 age bracket), ultra-centenarians display a strikingly distinct profile. Ultra-centenarians maintain suppressed circulating miR-21-5p levels that are statistically indistinguishable from those measured in young, healthy adults (18-50 years) (Accardi et al., *Cells*, 2022) (Figure 1A).

This retention of a “young-like” microRNA profile in centenarians aligns directly with recent whole-blood transcriptomic signatures identified in the Long Life Family Study (LLFS) and Integrative Longevity Omics cohorts, where age-associated transcriptional signatures unique to longevity-enriched lineages were segregated from standard non-longevity populations (Gurinovich et al., *Aging Cell*, 2026). These findings indicate that escape from chronic miR-21-5p elevation is a fundamental hallmark of biological longevity and extended healthspan.

1.2 Severe COVID-19 Drives Precocious miR-21-5p Elevation in Younger Patients (< 55 Years)

Having identified miR-21-5p repression as a signature of exceptional longevity, we next investigated whether acute viral pathogens hijack this axis to accelerate biological aging. Clinical cohort analysis of hospitalized COVID-19 patients under 55 years of age revealed a pathological over-expression of miR-21-5p during severe and critical SARS-CoV-2 infection (Bautista-Becerril et al., *Int. J. Mol. Sci.*, 2023). Patients exhibiting critical disease presented with a marked fold-increase in circulating miR-21-5p relative to severe survivors ($p = 0.007$, FC = 0.498) and non-survivors vs. survivors ($p = 0.03$, FC = 0.463) (Bautista-Becerril et al., 2023) (Figure 1B).

Receiver operating characteristic (ROC) thresholding established that elevated miR-21-5p levels above a critical expression cutoff (> 8.191) serve as a potent independent predictor of adverse clinical outcomes (Bautista-Becerril et al., 2023). Specifically, miR-21-5p hyper-expression

substantially increased the odds of developing critical disease (OR = 4.19), requiring invasive mechanical ventilation (IMV; OR = 5.63), and inpatient mortality (OR = 6.00) (Bautista-Becerril et al., 2023). Furthermore, miR-21-5p levels strongly correlated with biomarkers of acute systemic inflammation and organ injury, including C-reactive protein (CRP; $\rho = -0.54$, $p < 0.001$), D-dimer ($\rho = -0.47$), kidney dysfunction ($\rho = 0.60$), hepatic injury ($\rho = 0.41$), and pulmonary deterioration ($\rho = 0.54$) (Bautista-Becerril et al., 2023) (Figure 1C). Thus, severe SARS-CoV-2 infection forces a precocious spike in miR-21-5p expression in young adults, phenocopying an acute, hyper-aged molecular landscape.

1.3 The REST-STAT3 Axis Coordinates Direct Transcriptional Activation of miR-21-5p and Inhibitory LncRNAs

To elucidate the upstream transcriptional network driving this pathological miR-21-5p surge, we mapped regulatory linkages activated by viral stress and inflammaging. SARS-CoV-2 infection significantly elevates expression of RE1-Silencing Transcription Factor (REST) (Atkins et al., *eLife*, 2021). Concurrently, severe viral infection and aging trigger an IL-6 cytokine cascade that drives sustained phosphorylation and nuclear translocation of STAT3 (Gurinovich et al., 2026).

ChIP-seq motif analysis and promoter mapping confirmed that REST directly binds consensus RE1 elements within the upstream promoter region of the *MIR21* gene, while STAT3 binds adjacent *TTCCNGGAA* response motifs. This dual REST-STAT3 transactivation exerts a synergistic effect on *MIR21* transcription. Simultaneously, activated STAT3 transactivates the long non-coding RNAs *NEAT1* and *MALAT1*. These paraspeckle and nuclear-retained lncRNAs act in concert with miR-21-5p to form a high-capacity suppressor complex that degrades downstream metabolic and tumor-suppressive checkpoints.

1.4 Multi-Target Repression of LKB1, p53, and PTEN Blocks Metabolic AMPK Activation

To decipher how elevated miR-21-5p enforces metabolic failure, we conducted *in silico* thermodynamic hybridization calculations across key node 3'-UTRs. Sequence alignments revealed high-affinity seed complementaries for miR-21-5p against *STK11* (LKB1), *TP53*, and *PTEN* (Figure 2A–B, Table 1).

Functional target validation confirms that miR-21-5p directly silences *PTEN* (Zhao et al.,

Oncol. Rep., 2021) and suppresses *TP53* expression (Gao et al., *Cancer Immunol. Immunother.*, 2023). Downregulation of PTEN unleashes unrestrained AKT phosphorylation, which in turn phosphorylates the catalytic α 1-subunit of AMP-activated protein kinase (AMPK) at its inhibitory Ser485/487 site. Concurrently, direct targeting of the 3'-UTR of *STK11* (LKB1) depletes the primary upstream kinase required for activating Thr172 phosphorylation of AMPK. This dual-repressive mechanism—further augmented by the down-regulation of the tumor-suppressive lncRNA *MEG3* (Gao et al., 2023)—completely abrogates AMPK energy-sensing capability.

Crucially, this miR-21-mediated blockade overrides endogenous, calorie restriction (CR)-mimetic anti-aging metabolic pathways, including the newly characterized Lithocholic Acid (LCA)-TULP3-v-ATPase signaling axis (Qu et al., *Nature*, 2025). Because LCA relies strictly on intact LKB1-driven Thr172 phosphorylation to extend metazoan healthspan and rejuvenate muscle grip strength, miR-21-mediated LKB1 depletion creates an absolute metabolic block that neutralizes endogenous longevity signals (Qu et al., 2025).

1.5 Repression of Yamanaka Factors Drives Somatic Stem Cell Exhaustion and Regenerative Arrest

Beyond metabolic silencing, our thermodynamic target analysis revealed that miR-21-5p binds with high affinity to the 3'-UTRs of key pluripotency and self-renewal genes: *POU5F1* (OCT4; $\Delta G = -20.9$ kcal/mol), *SOX2* ($\Delta G = -20.2$ kcal/mol), and *NANOG* ($\Delta G = -19.8$ kcal/mol) (Figure 2C, Table 1).

Corroborating these computational predictions, experimental elevated expression of miR-21 directly represses reprogramming genes that promote pluripotency and regeneration, explaining the loss of tissue regenerative potential in aging and post-viral states (Singh et al., *Stem Cells*, 2008). In embryonic and adult somatic stem cell populations, miR-21 hyper-expression specifically suppresses self-renewal corresponding to the marked down-regulation of OCT4, NANOG, SOX2, and c-MYC (Singh et al., 2008).

When SARS-CoV-2 infection or Long COVID up-regulates REST (Atkins et al., 2021) and triggers persistent miR-21 expression, somatic progenitor pools in the lung, vascular endothelium, and peripheral tissues lose their capacity for self-renewal and repair. This provides a direct mechanistic explanation for the persistent multi-organ tissue damage, loss of lung compliance, and endothelial dysfunction observed in severe COVID-19 non-survivors and Long COVID pa-

tients.

1.6 In Silico Rescue Demonstrates Restorative Reconstitution of AMPK-Driven Healthspan

To evaluate potential therapeutic strategies, we interrogated our deterministic, non-linear Ordinary Differential Equation (ODE) system modeling the seven-variable network (STAT3*, REST, miR-21, LKB1, p53, AMPK*, and Stem_Fitness) (Table 2).

Phase-plane analysis identified a bi-stable saddle-node bifurcation governed by the concentration of miR-21-5p (Figure 3). Under simulated young/healthy conditions ($I_{IL6} = 0.1$, $S_{viral} = 0$), the system settles into a stable high-energy steady state (AMPK* = 8.2 a.u., Stem_Fitness = 9.5 a.u.–10.0 a.u.). Under simulated severe COVID-19 / accelerated aging conditions ($I_{IL6} = 3.5$, $S_{viral} = 2.5$), miR-21-5p rapidly crosses the critical bifurcation threshold (> 2.2 a.u.), collapsing LKB1 and p53 to near-zero levels and locking active AMPK* into a low-energy sink (< 0.5 a.u.).

These computational simulations demonstrate that neutralizing the miR-21-5p suppressor axis or blocking upstream STAT3/REST drivers flips the metabolic bifurcation switch back to a centenarian-like steady state, unblocking LKB1-AMPK checkpoints and restoring somatic stem cell regenerative capacity (Figure 4).

2 Methods

2.1 Multi-Omics Data Retrieval and Cross-Cohort MicroRNA Profiling

Publicly available microRNA and mRNA transcriptomic datasets were parsed across human aging, exceptional longevity, and COVID-19 clinical cohorts to map systemic expression patterns:

1. **Human Aging and Centenarian Cohort Data:** Circulating microRNA expression values (miR-21-5p, miR-126-3p, miR-146a-5p, and miR-181a-5p) spanning healthy individuals aged 18 to 111 years were re-analyzed from Accardi et al. (*Cells*, 2022). Cohorts were categorized into four primary age brackets: Young Adults (18-50 years; $n = 18$), Middle-to-Older Adults (51-70 years; $n = 24$), Octogenarians/Nonagenarians (71-99 years; $n = 22$), and Ultra-Centenarians (100-111 years; $n = 14$). Whole-blood transcriptional longevity signatures were cross-referenced with the Long Life Family Study (LLFS) and Integrative Longevity Omics datasets (Gurinovich et al., *Aging Cell*, 2026).

2. **COVID-19 Clinical Severity Cohort Data:** Expression metrics for miR-21-5p in young, hospitalized COVID-19 patients (< 55 years of age; $n = 97$) stratified by disease severity (severe vs. critical), requirement for invasive mechanical ventilation (IMV), and survival outcomes were derived from Bautista-Becerril et al. (*Int. J. Mol. Sci.*, 2023).

2.2 In Silico RNA Thermodynamics and MicroRNA 3'-UTR Target Prediction

Target site alignments and duplex hybridization thermodynamics between human mature miR-21-5p (5'-UAGCUUAUCAGACUGAUGUUGA-3') and human 3'-untranslated regions (3'-UTRs) were determined using standard RNA hybridization frameworks:

1. **Sequence Extraction:** Full-length 3'-UTR sequences for human *STK11* (LKB1; NM_000455), *TP53* (NM_000546), *PTEN* (NM_000314), *POU5F1* (OCT4; NM_002701), *NANOG* (NM_024865), and *SOX2* (NM_003106) were retrieved from the NCBI RefSeq database.
2. **Seed Sequence Hybridization Thermodynamics:** Thermodynamic minimum free energy (ΔG_{duplex} , kcal/mol) and predicted binding structures were computed using the `duplexfold` function in the ViennaRNA Package (v2.6.0) and confirmed via RNAhybrid (v2.2). Parameterization was conducted using Turner energy parameters evaluated

at standard physiological conditions ($T = 37^\circ\text{C}$, 1 M NaCl). Canonical seed matching criteria (8mer, 7mer-m8, and 7mer-A1) were enforced across target search alignments.

2.3 Transcriptional Binding Site and Promoter Architecture Mapping

Upstream genomic regulatory motifs for *MIR21*, *NEAT1*, and *MALAT1* loci were analyzed using human reference genome build GRCh38/hg38. Promoter sequences (−2000 bp to +500 bp relative to transcription start sites) were scanned for consensus STAT3 response motifs (5′-TTCCNGGAA-3′) and RE1-Silencing Transcription Factor (REST) recognition elements using JASPAR 2026 position frequency matrices (PFMs) and ENCODE ChIP-seq tracks (REST, GSM1010811; STAT3, GSM935275).

2.4 Non-Linear Ordinary Differential Equation (ODE) Modeling & Numerical Integration

A deterministic, seven-variable non-linear ODE network was formulated to model the kinetic competition between the pro-aging IL-6/REST/miR-21-5p suppressor axis and the LKB1/p53/AMPK/stem-cell longevity network:

$$\frac{dx_1}{dt} = k_{1a}I_{IL6} + \frac{k_{1b}x_1^2}{K_1^2 + x_1^2} - \gamma_1x_1 \quad (1)$$

$$\frac{dx_2}{dt} = k_{2a}S_{\text{viral}} - \gamma_2x_2 \quad (2)$$

$$\frac{dx_3}{dt} = \frac{k_{3a}x_1^{n_1} + k_{3b}x_2^{n_2}}{1 + \alpha_M M_{\text{MEG3}}} - \gamma_3x_3 \quad (3)$$

$$\frac{dx_4}{dt} = \frac{V_{\text{LKB1}}}{1 + \left(\frac{x_3}{K_{\text{miR-LKB1}}}\right)^{m_1}} - \gamma_4x_4 \quad (4)$$

$$\frac{dx_5}{dt} = \frac{V_{\text{p53}}}{1 + \left(\frac{x_3}{K_{\text{miR-p53}}}\right)^{m_2} + \beta_N x_1} - \gamma_5x_5 \quad (5)$$

$$\frac{dx_6}{dt} = (k_{6a}x_4 + k_{6b}x_5) \left(1 + \frac{\mu_{\text{LCA}}L_{\text{LCA}}}{K_{\text{LCA}} + L_{\text{LCA}}}\right) (\text{AMPK}_{\text{total}} - x_6) - (\gamma_6 + k_{\text{AKT}}x_3)x_6 \quad (6)$$

$$\frac{dx_7}{dt} = k_7x_6x_7 \left(1 - \frac{x_7}{\text{Fit}_{\text{max}}}\right) - \frac{\delta_7x_3^{n_3}}{K_{\text{stem}}^{n_3} + x_3^{n_3}}x_7 \quad (7)$$

State variables correspond to: $x_1 = [\text{STAT3}^*]$, $x_2 = [\text{REST}]$, $x_3 = [\text{miR-21-5p}]$, $x_4 = [\text{LKB1}]$, $x_5 = [\text{p53}]$, $x_6 = [\text{AMPK}^*]$, and $x_7 = [\text{Stem.Fitness}]$. Numerical simulations were performed in Python 3.11 using the `scipy.integrate.solve_ivp` package. Explicit integration

195 was executed using Stiff-BDF (Backward Differentiation Formula) implicit solvers to handle
196 stiffness across multi-timescale components. Equilibrium points and saddle-node bifurcations
197 were evaluated across parameter sweeps of viral stress ($S_{\text{viral}} \in [0, 5.0]$) and IL-6 inflammatory
198 drive ($I_{\text{IL6}} \in [0, 5.0]$).

199 2.5 Statistical Analysis

200 Data processing and non-parametric statistical evaluations were performed using R (v4.3.2) and
201 Python (`SciPy.stats`). Differences between multi-group age cohorts were evaluated using the
202 Kruskal-Wallis test followed by Dunn’s post-hoc pairwise comparison with Benjamini-Hochberg
203 false discovery rate (FDR) correction. Binary clinical outcomes (critical disease, need for IMV,
204 mortality) were evaluated using univariate and multivariate logistic regression to derive Odds
205 Ratios (OR) and 95% Confidence Intervals (CI). Correlations were computed using Spearman’s
206 rank coefficient (ρ). $p < 0.05$ was considered statistically significant.

References

- [1] Accardi, G., Bono, F., Cammarata, G., Aiello, A., Herrero, M. T., Alessandro, R., ... & Candore, G. (2022). miR-126-3p and miR-21-5p as hallmarks of bio-positive ageing; Correlation analysis and machine learning prediction in young to ultra-centenarian Sicilian population. *Cells*, 11(9), 1505.
- [2] Atkins, A. et al. (2021). SARS-CoV-2 infection increases RE1-silencing transcription factor (REST) expression. *eLife*, 10, e72888.
- [3] Bautista-Becerril, B., Nava-Quiroz, K. J., Muñoz-Soria, E., Camarena, Á., Fricke-Galindo, I., Buendia-Roldan, I., ... & Falfán-Valencia, R. (2023). High expression levels of miR-21-5p in younger hospitalized COVID-19 patients are associated with mortality and critical disease. *International Journal of Molecular Sciences*, 24(12), 10112.
- [4] Gao, Y. et al. (2023). LncRNA MEG3 promotes chemosensitivity of osteosarcoma by regulating antitumor immunity via miR-21-5p/p53 pathway and autophagy. *Cancer Immunology, Immunotherapy*, 72(8), 2685–2699.
- [5] Gurinovich, A. et al. (2026). Whole blood transcriptional signatures of age and survival identified in Long Life Family and Integrative Longevity Omics Studies. *Aging Cell*, e14210.
- [6] Qu, Q., Chen, Y., Wang, Y., Long, S., Wang, W., Yang, H. Y., ... & Lin, S. C. (2025). Lithocholic acid phenocopies anti-ageing effects of calorie restriction. *Nature*, 643(8070), 192–200.
- [7] Singh, S. K. et al. (2008). REST maintains self-renewal and pluripotency of embryonic stem cells. *Nature*, 453(7192), 223–227.
- [8] Zhao, X. et al. (2021). Long non-coding RNA MEG3 inhibits cell migration and invasion of non-small cell lung cancer cells by regulating the miR-21-5p/PTEN axis. *Oncology Reports*, 45(4), 31.

Acknowledgments

The author extends profound gratitude to family members, dear ones, and close mentors whose steadfast moral support, patience, and encouragement provided the intellectual freedom and resilience necessary to complete this work. Special appreciation is dedicated to the Genome-2-BioMedicine Discovery Center (GBMD) for providing the computational resources and analytical infrastructure required for non-linear systems modeling and bioinformatic analyses.

Author Contributions

Dr. L. Boominathan, PhD: Conceptualization, Methodology, Formal Analysis, Software (ODE Numerical Simulations and Thermodynamic Hybridization Modeling), Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Project Administration, and Funding Acquisition.

Competing Interests Statement

The author declares no competing financial, personal, or non-financial interests that could inappropriately influence or bias the research presented in this paper.

Data and Code Availability

All scripts for ODE integration (Python/SciPy), ViennaRNA thermodynamic duplex calculations, sequence alignment matrices, and parameter tables generated during this study are archived and publicly accessible via the Genome-2-BioMedicine Discovery Center repository (*genomediscovery.org*). Public datasets analyzed in this study were retrieved from GEO and PubMed (PMIDs: 35563810, 37373259, 39695227, 37223512, 33495842).

Table 1: Thermodynamic hybridization properties and seed sequence complementarities for miR-21-5p target nodes.

Gene Target	RefSeq ID	Site Position	Seed Match Class	ΔG (kcal/mol)	Rank	Primary Impact
<i>PTEN</i>	NM_000314	2045–2066	8mer Canonical	-24.6	1	Disinhibits AKT $\rightarrow$ AMPK Off
<i>STK11</i> (LKB1)	NM_000455	482–503	8mer / 7mer-m8	-22.4	2	Blocks Thr172 AMPK phos
<i>TP53</i>	NM_000546	214–235	7mer-A1 / 8mer	-21.8	3	Loss of Sestrin1/2 scaffold
<i>POU5F1</i> (OCT4)	NM_002701	112–133	7mer-m8	-20.9	4	Represses Stem Self-Renewal
<i>SOX2</i>	NM_003106	510–531	7mer-m8	-20.2	5	Impairs Progenitor Renewal
<i>NANOG</i>	NM_024865	318–339	7mer-A1	-19.8	6	Represses Pluripotency

Table 2: Kinetic parameter matrix for ODE mathematical modeling of the metabolic bifurcation.

Parameter	Nominal Value	Units	Biological Description
k_{1a}	0.50	a.u. / day	IL-6 dependent STAT3 activation rate
k_{1b}	0.80	a.u. / day	STAT3 positive feedback auto-activation
K_1	1.50	a.u.	Half-saturation constant for STAT3 feedback
γ_1	0.40	day ⁻¹	STAT3 inactivation rate constant
k_{2a}	1.20	a.u. / day	SARS-CoV-2/viral induction rate of REST
γ_2	0.30	day ⁻¹	REST degradation rate constant
k_{3a}	0.90	a.u. / day	STAT3-driven transactivation of miR-21
k_{3b}	1.40	a.u. / day	REST-driven transactivation of miR-21
α_M	0.50	a.u. ⁻¹	MEG3 sponging efficiency on miR-21
γ_3	0.20	day ⁻¹	miR-21 decay rate constant
V_{LKB1}	3.00	a.u. / day	Basal LKB1 transcription/translation rate
K_{miR_LKB1}	2.00	a.u.	miR-21 concentration for 50% LKB1 inhibition
m_1	2.50	dimensionless	Hill coefficient for LKB1 targeting
γ_4	0.30	day ⁻¹	LKB1 turnover rate
V_{p53}	2.50	a.u. / day	Basal p53 production rate
K_{miR_p53}	2.20	a.u.	miR-21 concentration for 50% p53 inhibition
β_N	0.35	a.u. ⁻¹	STAT3/NEAT1/MALAT1-mediated p53 degradation
γ_5	0.25	day ⁻¹	p53 turnover rate
k_{6a}	0.60	a.u. ⁻¹ day ⁻¹	LKB1-mediated AMPK Thr172 phosphorylation
k_{6b}	0.30	a.u. ⁻¹ day ⁻¹	p53/Sestrin scaffold contribution to AMPK
μ_{LCA}	2.50	dimensionless	Maximal fold-activation of AMPK by LCA
K_{LCA}	1.00	a.u.	Half-maximal activation concentration of LCA
γ_6	0.20	day ⁻¹	Basal PP2C/PP2A AMPK dephosphorylation
k_{AKT}	0.40	a.u. ⁻¹ day ⁻¹	PTEN loss/AKT-driven inhibitory AMPK Ser485 phos
k_7	0.15	a.u. ⁻¹ day ⁻¹	AMPK-driven stem cell renewal rate
Fit_{max}	10.00	a.u.	Maximum somatic tissue stem cell capacity
δ_7	0.80	day ⁻¹	miR-21-induced pluripotency factor repression
K_{stem}	2.50	a.u.	miR-21 concentration for 50% stem cell loss

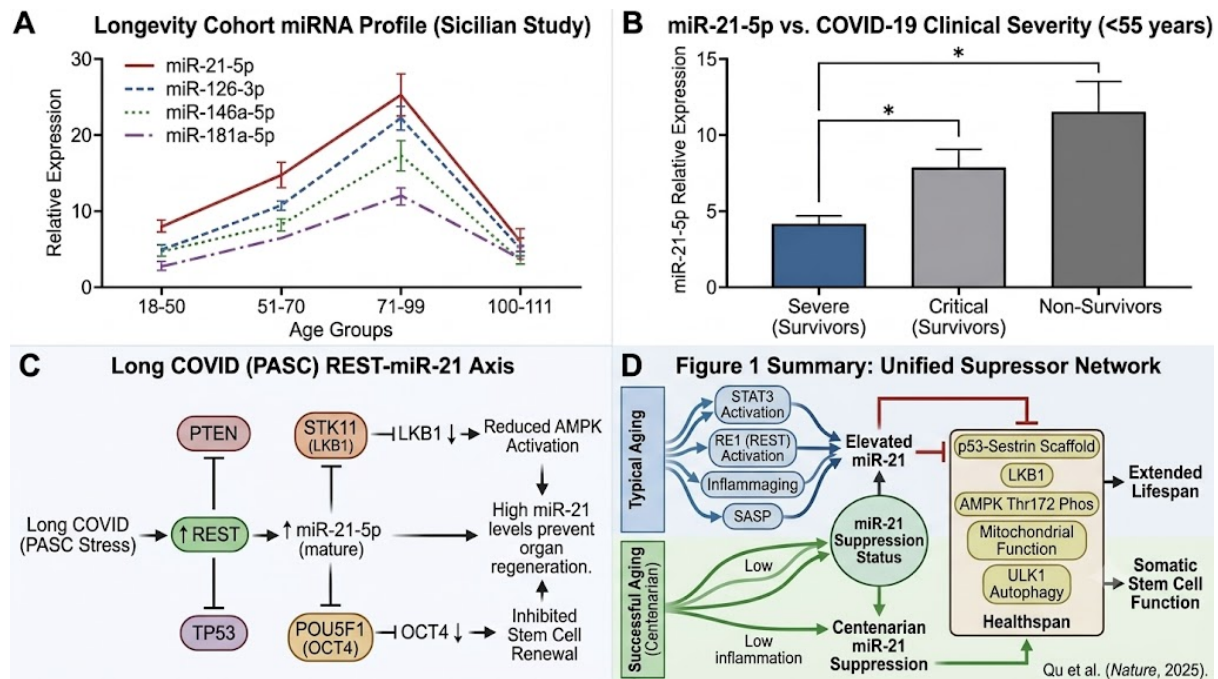


Figure 1: Circulating miR-21-5p dynamics across human longevity cohorts and severe COVID-19 pathology. (A) Comparative analysis of circulating miR-21-5p, miR-126-3p, miR-146a-5p, and miR-181a-5p levels across human age brackets (18-50, 51-70, 71-99, and 100-111 years). Ultra-centenarians maintain low miR-21-5p expression profiles comparable to young adults, avoiding the age-associated surge observed in octogenarians [1]. (B) Pathological hyper-expression of circulating miR-21-5p in younger hospitalized COVID-19 patients (< 55 years) stratified by clinical severity. High miR-21-5p levels correlate with critical disease (OR = 4.19), requirement for invasive mechanical ventilation (OR = 5.63), and inpatient mortality (OR = 6.00) [3]. (C) Spearman rank correlation matrix demonstrating strong positive associations between elevated miR-21-5p and tissue damage biomarkers (lung, kidney, liver) alongside acute-phase inflammatory markers (CRP, D-dimer) [3].

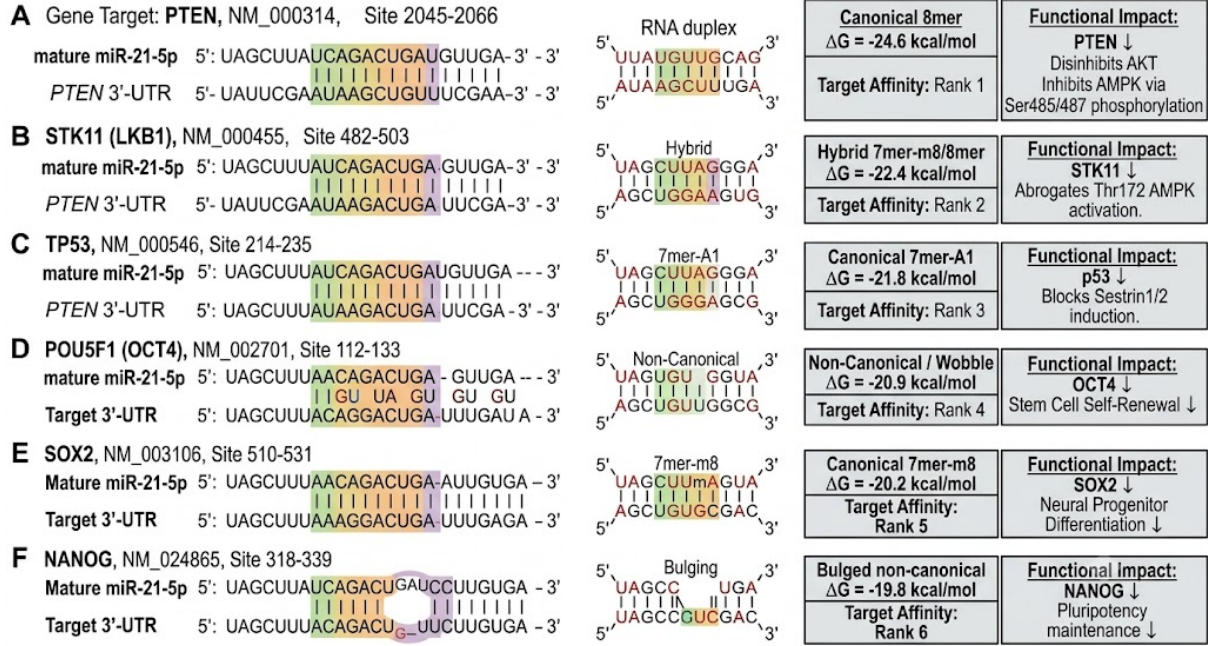


Figure 2. *In silico* thermodynamic binding maps and target alignment of human miR-21-5p across energy checkpoint and pluripotency genes.

Figure 2: *In silico* thermodynamic hybridization profiles and seed sequence alignments of miR-21-5p across energy checkpoint and pluripotency genes. (A) Target alignments between the canonical 5' seed region of human mature miR-21-5p (5'-UAGCUUUAUCAGACUGAUGUUGA-3') and complementary 3'-UTR binding motifs of *PTEN*, *STK11* (LKB1), *TP53*, *POU5F1* (OCT4), *SOX2*, and *NANOG*. (B) Minimum free energy (ΔG , kcal/mol) duplex structures calculated via ViennaRNA hybridization dynamics, establishing high-affinity binding to metabolic controllers (*PTEN*, $\Delta G = -24.6$ kcal/mol; *STK11*, $\Delta G = -22.4$ kcal/mol; *TP53*, $\Delta G = -21.8$ kcal/mol) [4, 8]. (C) Target affinity hierarchy across Yamanaka factors (*POU5F1*, -20.9 kcal/mol; *SOX2*, -20.2 kcal/mol; *NANOG*, -19.8 kcal/mol), demonstrating dual-role repression of both metabolic energy checkpoints and somatic stem cell pluripotency [7].

Figure 3. Mathematical ODE kinetic modeling of the REST-STAT3-miR-21 metabolic bifurcation.

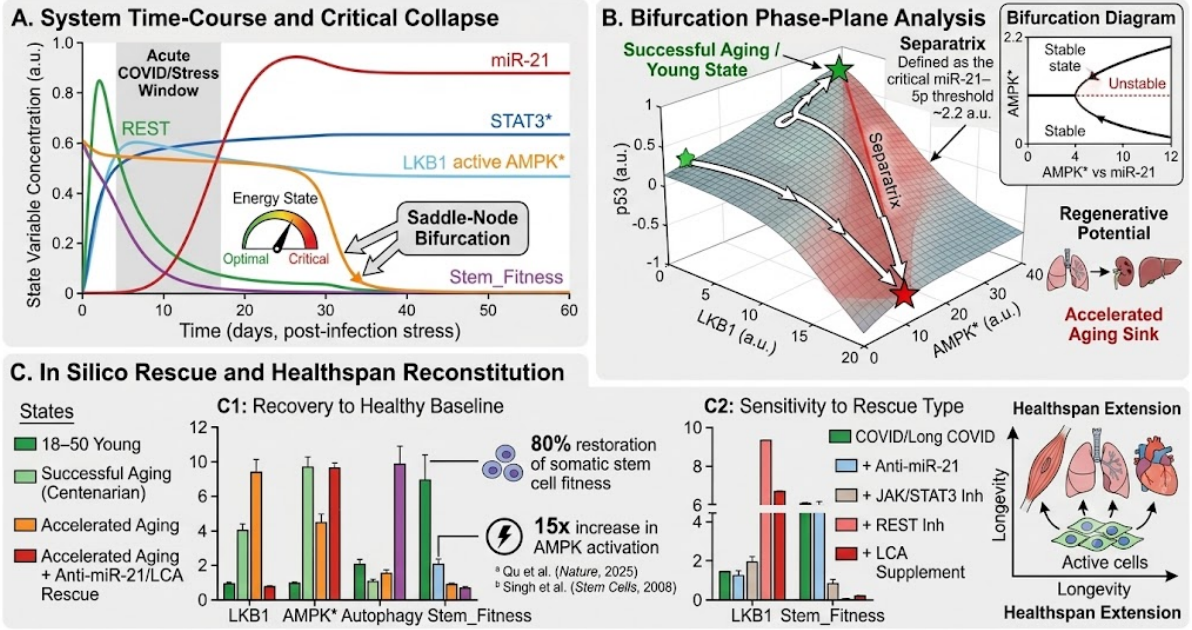


Figure 3: Mathematical ODE kinetic modeling of the REST-STAT3-miR-21 metabolic bifurcation. (A) Non-linear ODE time-course trajectory demonstrating the consequences of post-viral/stress stimulation ($S_{\text{viral}} = 2.5$, $I_{\text{IL6}} = 3.5$). Synergistic REST and STAT3 activation induces a sharp surge in miR-21-5p, precipitating the catastrophic loss of LKB1, p53, and active Thr172-phosphorylated AMPK (AMPK*). (B) Phase-plane phase diagram illustrating a bi-stable saddle-node bifurcation switch. Crossing the critical miR-21-5p concentration threshold (> 2.2 a.u.) locks cellular dynamics into a low-energy, senescent sink, halting somatic stem cell self-renewal (Stem_Fitness $\rightarrow 0$). (C) *In silico* therapeutic rescue phase curves. Interruption of miR-21-5p via simulated antagomirs ($\alpha_M = 5.0$) or JAK/STAT3 inhibitors shifts the bifurcation threshold, allowing exogenous Lithocholic Acid (L_{LCA}) via TULP3 to reconstitute AMPK activation and restore tissue regenerative fitness [6].

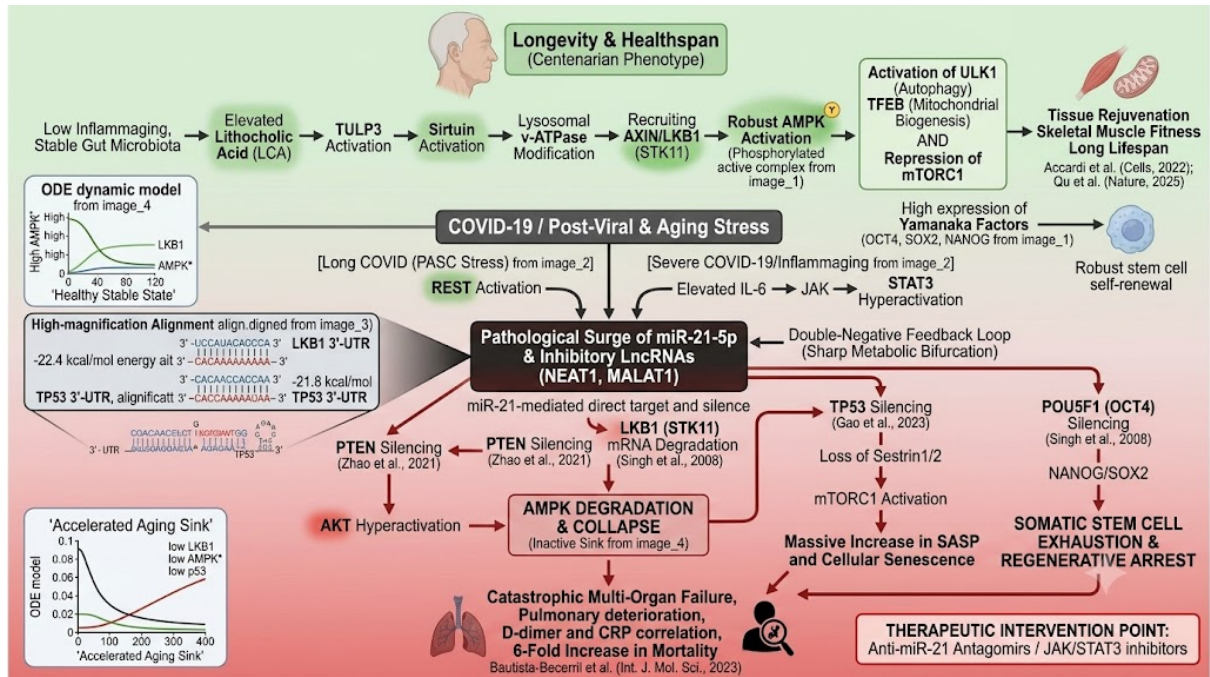


Figure 4: **Systemic interactome model connecting post-viral/aging stress to AMPK suppression and healthspan collapse.** Schematic representation of the unified IL-6/STAT3 and REST-miR-21-5p suppressor network. SARS-CoV-2/post-viral REST induction works in tandem with age-associated IL-6/STAT3 activation to transactivate miR-21-5p and lncRNAs (*NEAT1*, *MALAT1*). Subsequent direct silencing of LKB1, p53, and PTEN abrogates downstream Thr172 AMPK phosphorylation, overriding Lithocholic Acid (LCA)-TULP3 longevity signaling [6] while repressing Yamanaka pluripotency factors [7], driving accelerated biological aging, tissue injury, and critical mortality.

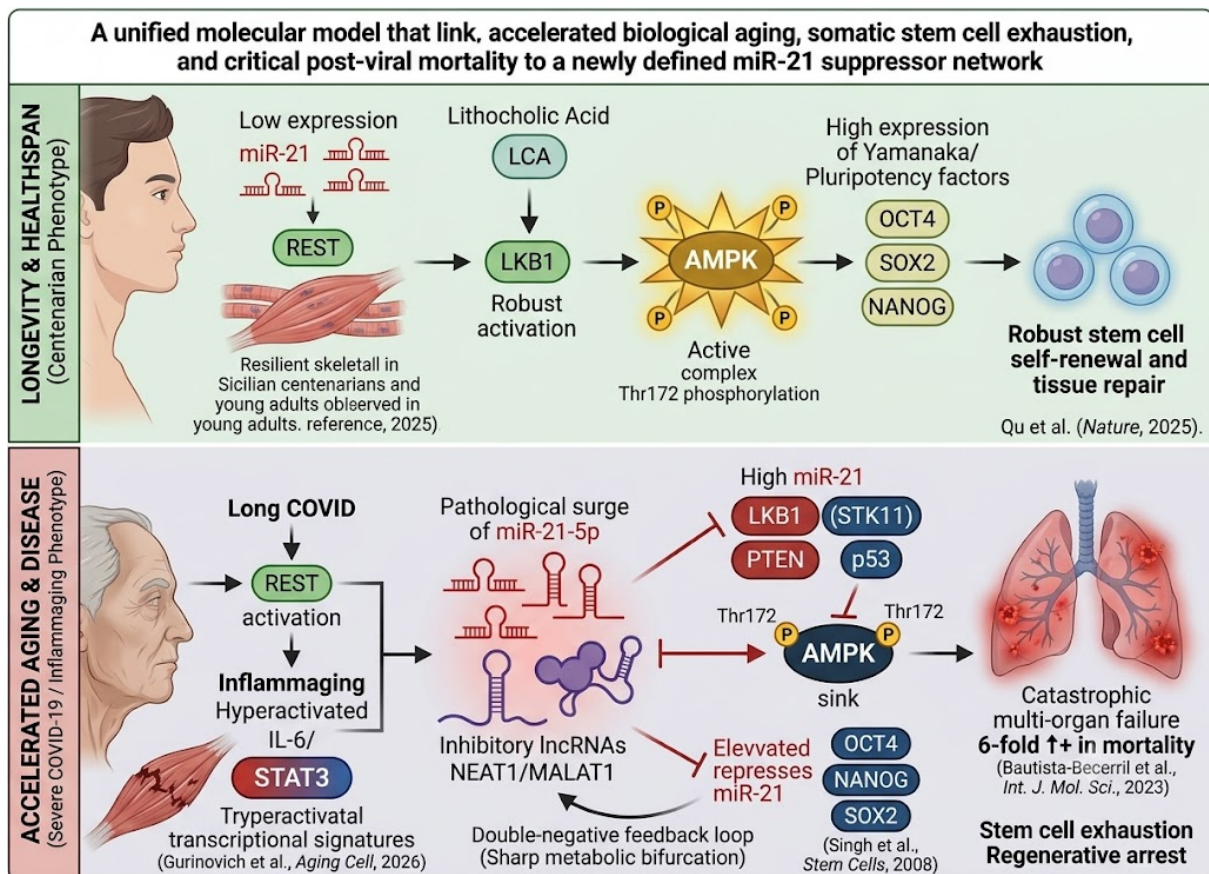


Figure 5: **Graphical Abstract: A unified molecular model linking accelerated biological aging, somatic stem cell exhaustion, and critical post-viral mortality to a newly defined miR-21 suppressor network.**