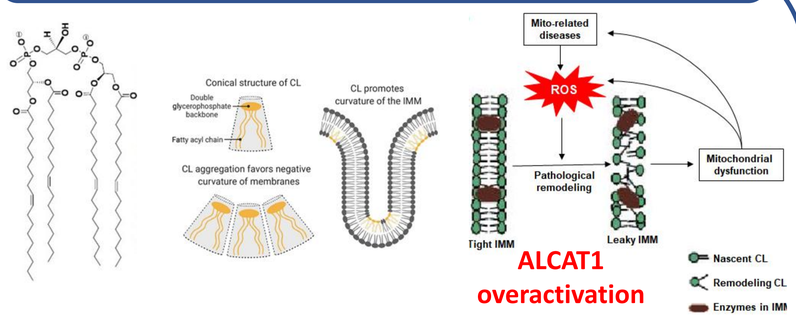
A cardiolipin-targeting α-helical bundle peptide stabilizes inner membrane of mitochondria, down-regulating ALCAT1 to shut down pathogenic remodeling in oxidative stress condition

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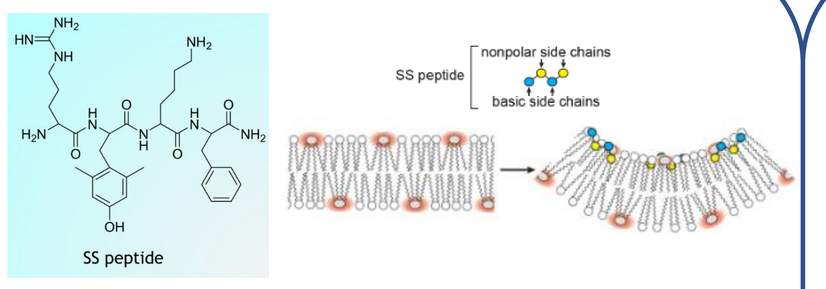
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Background: ROS-driven pathological cardiolipin (CL) remodeling destabilizes the IMM



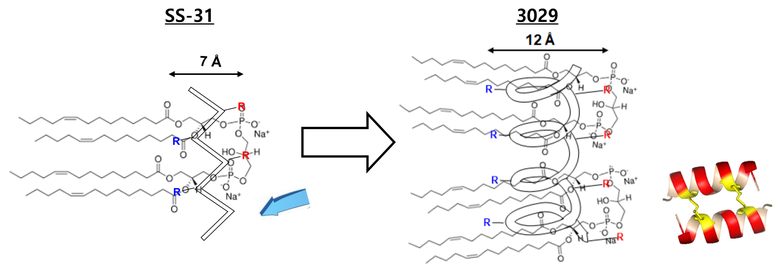
- **Essential role of CL:** Maintains IMM negative curvature and cristae stability via its unique conical structure.
- **ALCAT1 overactivation:** Oxidative stress (ROS) up-regulates ALCAT1, driving pathological CL remodeling.
- **IMM destabilization:** Altered CL composition transforms a healthy, "tight" IMM into a "leaky" membrane.
- **The vicious cycle:** Leaky IMM disrupts the electron transport chain (ETC), amplifying ROS production and mitochondrial diseases.

Proof of concept: SS-31 as a first-in-class CL-targeting peptide



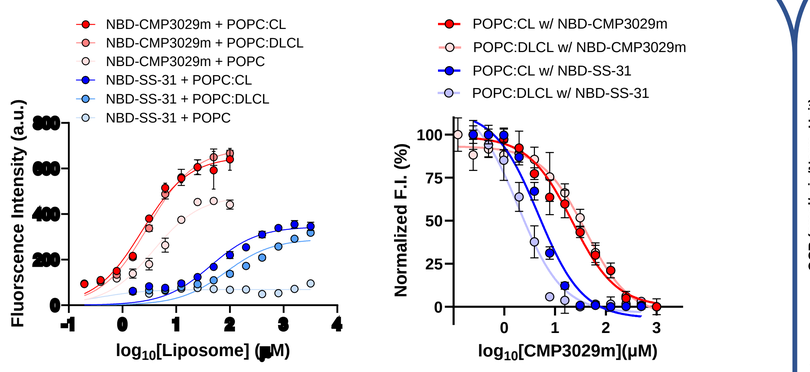
- **Selective CL binding:** SS-31 is a representative tetrapeptide that specifically binds to CL via alternating electrostatic and hydrophobic interactions.
- **Curvature protection:** It localizes to the IMM surface, maintaining structural curvature and preventing ROS-induced CL degradation.
- **Validation of target:** The established efficacy of SS-31 proves that stabilizing CL is a highly viable approach to treating mitochondrial dysfunction.

3029 vs. SS-31: α-Helical structure makes strong interaction w/ multiple CL binding



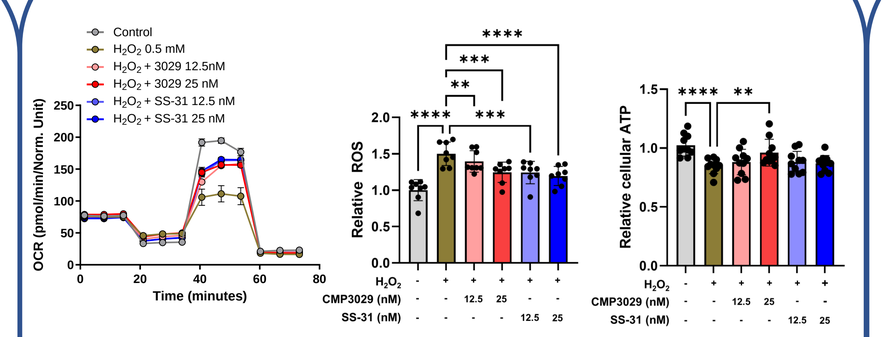
- **Broader interface:** α-helical **3029** provides a wider binding surface (~12 Å) than the short, β-sheet-like **SS-31** (~7 Å).
- **Multiplexed binding:** 3029 optimally arrays multiple Arginine (R) residues to cross-link multiple CL molecules simultaneously.
- **Superior stabilization:** This multi-point electrostatic binding offers significantly stronger IMM structural support compared to SS-31.

In vitro validation: 3029 exhibits stronger CL binding than SS-31



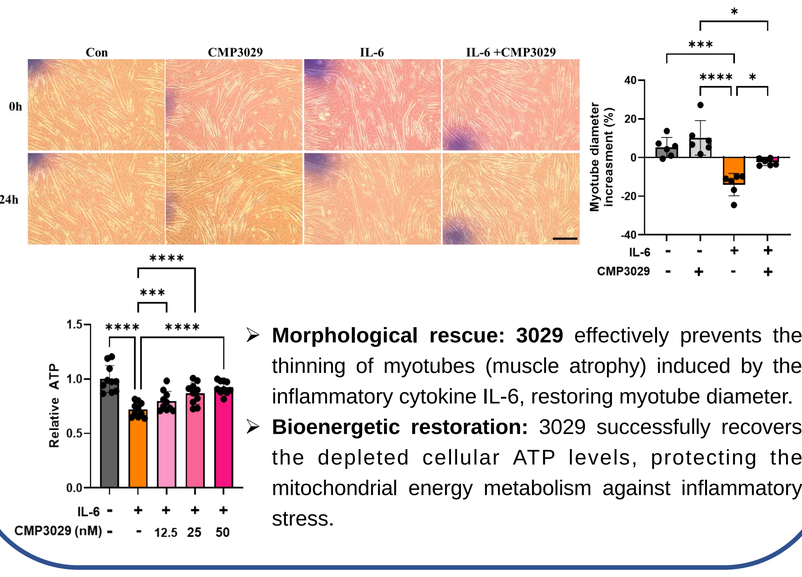
- **Enhanced affinity: 3029** demonstrates significantly stronger binding to CL/DLCL-containing liposomes compared to the benchmark **SS-31**.
- **Higher capacity:** The drastically elevated fluorescence intensity indicates a maximized insertion and interaction of 3029 into CL-rich membranes.
- **Tighter interaction:** Displacement assays confirm that 3029 forms a highly stable and robust complex with CL, slower dissociation than SS-31.

3029 vs. SS-31: Protection against oxidative stress



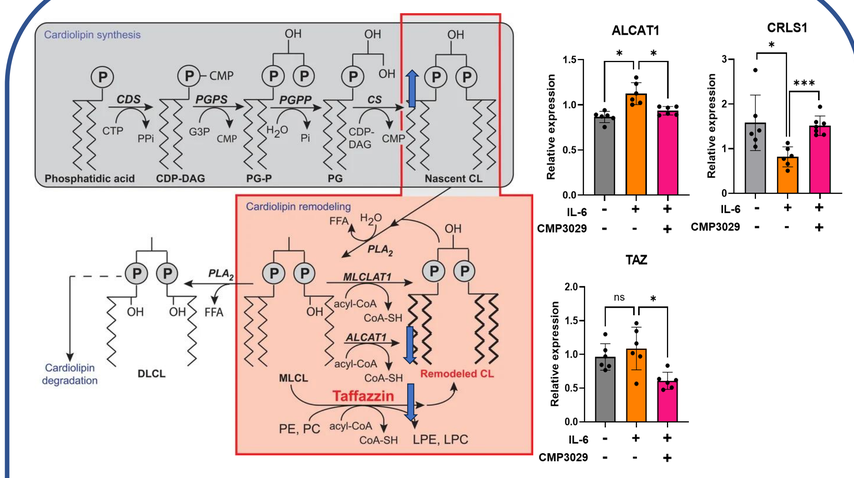
- **Respiration recovery: 3029** efficiently restores the Oxygen Consumption Rate (OCR) against H_2O_2-induced oxidative stress.
- **ROS suppression:** While **SS-31 exhibits a slightly stronger direct ROS scavenging effect**, 3029 still significantly reduces intracellular ROS accumulation.
- **Superior ATP recovery:** Notably, despite moderate ROS quenching, **3029 (25 nM) achieves better ATP restoration than SS-31**. This highlights that its robust structural IMM stabilization ultimately translates to superior bioenergetic protection.

3029 prevents IL-6-Induced muscle atrophy in C2C12 myotubes



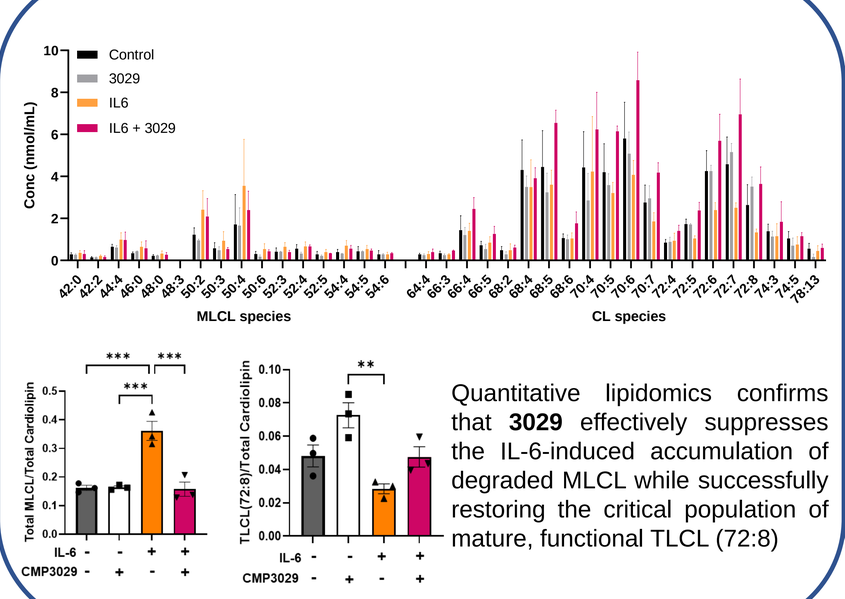
- **Morphological rescue: 3029** effectively prevents the thinning of myotubes (muscle atrophy) induced by the inflammatory cytokine IL-6, restoring myotube diameter.
- **Bioenergetic restoration:** 3029 successfully recovers the depleted cellular ATP levels, protecting the mitochondrial energy metabolism against inflammatory stress.

Mechanism of action: 3029 shuts down pathological CL remodeling



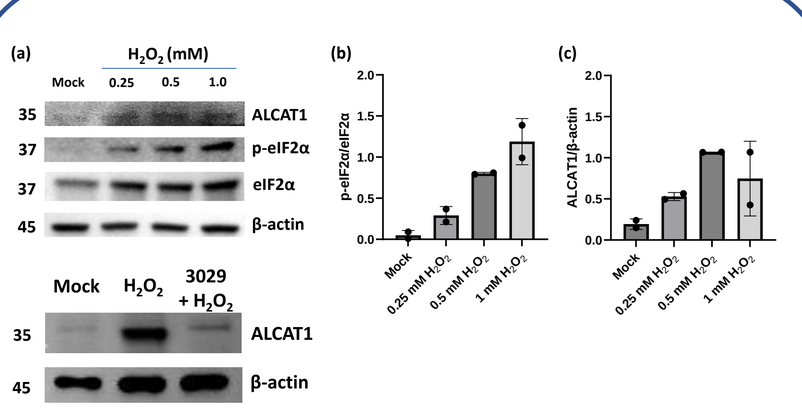
- Mechanistically, **3029** preserves mitochondrial integrity by simultaneously down-regulating the pathological remodeling enzyme **ALCAT1** and restoring the healthy nascent CL synthase **CRLS1** against IL-6-induced stress.

Lipidomic validation: 3029 restores healthy cardiolipin composition in C2C12 myotubes



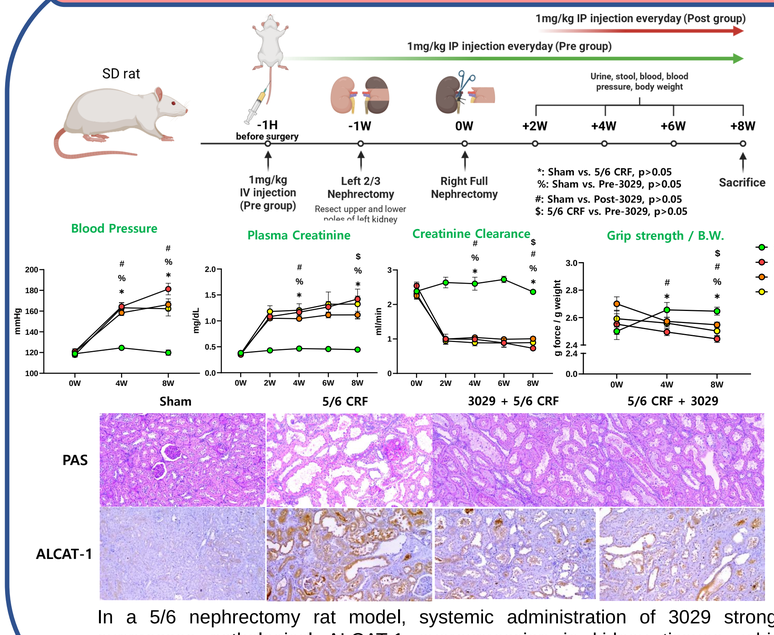
Quantitative lipidomics confirms that **3029** effectively suppresses the IL-6-induced accumulation of degraded MLCL while successfully restoring the critical population of mature, functional TLCL (72:8)

Inhibitory effect of 3029 on H_2O_2-induced ER Stress and ALCAT1 expression in HK-2 cells



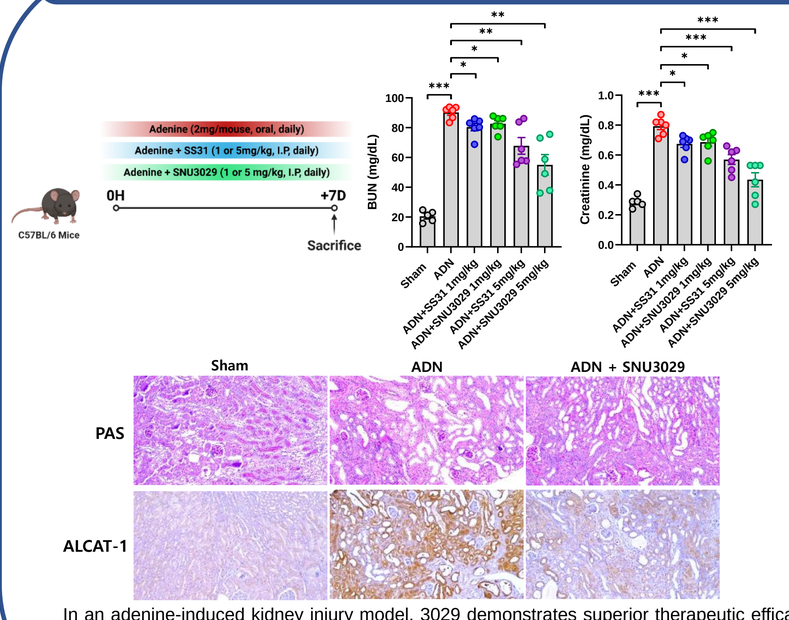
- Upregulation of ALCAT1 by H_2O_2: Concurrently with the elevation of p-eIF2α, ALCAT1 expression level also markedly increased in proportion to the H_2O_2 concentration.
- Inhibitory efficacy of 3029: Compared to the group treated with H_2O_2 alone, co-treatment with 3029 effectively suppressed the H_2O_2-induced abnormal overexpression of ALCAT1.

In vivo validation: 3029 ameliorates renal failure by suppressing ALCAT-1 in 5/6 CRF model rat



In a 5/6 nephrectomy rat model, systemic administration of 3029 strongly suppresses pathological ALCAT-1 overexpression in kidney tissues, which directly translates to mitigated renal dysfunction, normalized blood pressure, and prevention of muscle weakness.

Comparative *in vivo* efficacy: 3029 exhibits superior protection over SS-31 in adn-induced model mouse



In an adenine-induced kidney injury model, 3029 demonstrates superior therapeutic efficacy compared to SS-31 by more robustly suppressing structural tissue damage (PAS) and pathological ALCAT-1 overexpression, leading to a significantly greater reduction in renal dysfunction markers (BUN and Creatinine).

Conclusion: 3029 overcomes pathological CL remodeling

- **Structural superiority:** The optimized α-helical structure of 3029 enables multiplexed and maximized cardiolipin (CL) binding, providing fundamentally stronger inner mitochondrial membrane (IMM) stabilization than SS-31.
- **Pathological reversal:** 3029 successfully restores mitochondrial bioenergetics and healthy lipid profiles by effectively shutting down the ALCAT1-mediated pathological CL remodeling pathway.
- **Next-generation efficacy:** Demonstrating superior therapeutic outcomes over the benchmark SS-31 in treating *in vivo* renal failure and muscle atrophy, 3029 emerges as a highly promising next-generation candidate for mitochondria-targeted diseases.

References

1) Shin, G., Hyun, S., Kim, D., Choi, Y., Kim, K. H., Kim, D., ... & Yu, J. (2023). Cyclohexylalanine-containing α-helical amphipathic peptide targets cardiolipin, rescuing mitochondrial dysfunction in kidney injury. Journal of Medicinal Chemistry, 67(5), 3385-3399.