Literature Report

Reporter: yingzhu chen

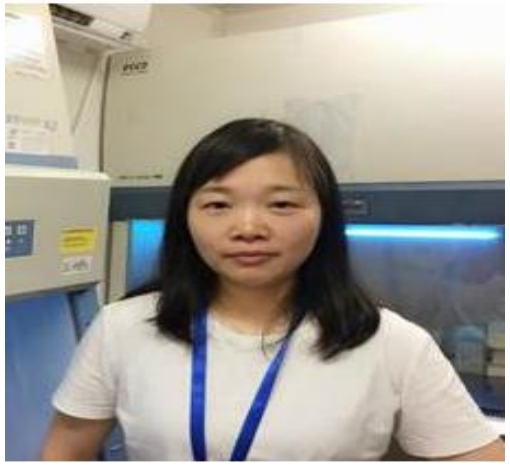
Date: 2021-08-05

Literature Source

ARTICLES
https://doi.org/10.1038/s41556-021-00710-0
nature cell biology
Check for updates

Targeting liquid–liquid phase separation of SARS-CoV-2 nucleocapsid protein promotes innate antiviral immunity by elevating MAVS activity

Shuai Wang[1,6], Tong Dai[1,6], Ziran Qin[1,6], Ting Pan[2,6], Feng Chu[1], Lingfeng Lou[1], Long Zhang[3], Bing Yang[3,4], Huizhe Huang[5], Huasong Lu[3] and Fangfang Zhou[1]✉



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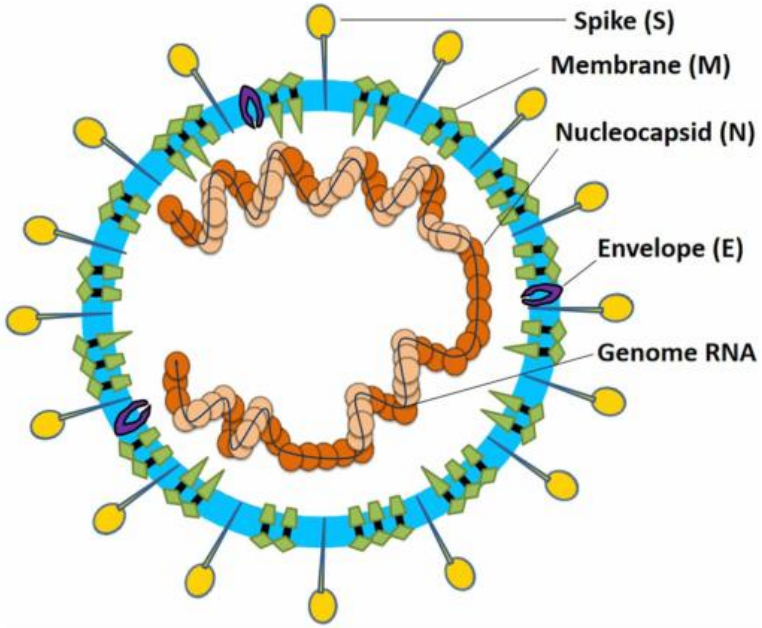
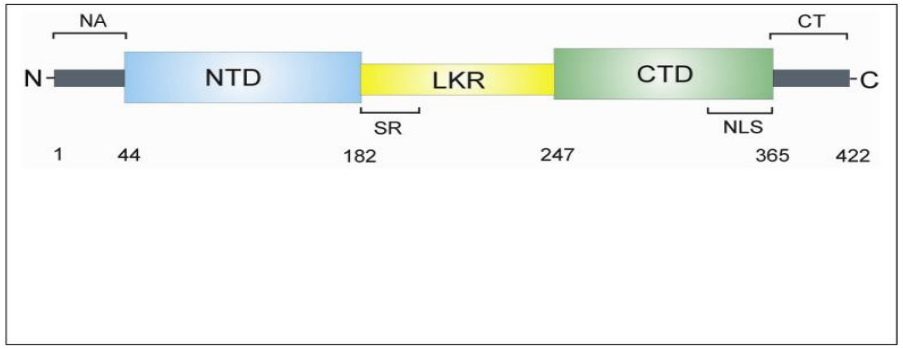
2008年在清华大学生物科学与技术系获得博士学位。2008-2013年在荷兰莱顿大学医学中心从事博士后研究。
2013-2014年在荷兰莱顿大学医学中心任研究助理教授。
2014年10月受聘于苏州大学医学部生物医学研究院。
研究方向：细胞信号转导、肿瘤与免疫的互作及分子机制等方面的研究

Introduction

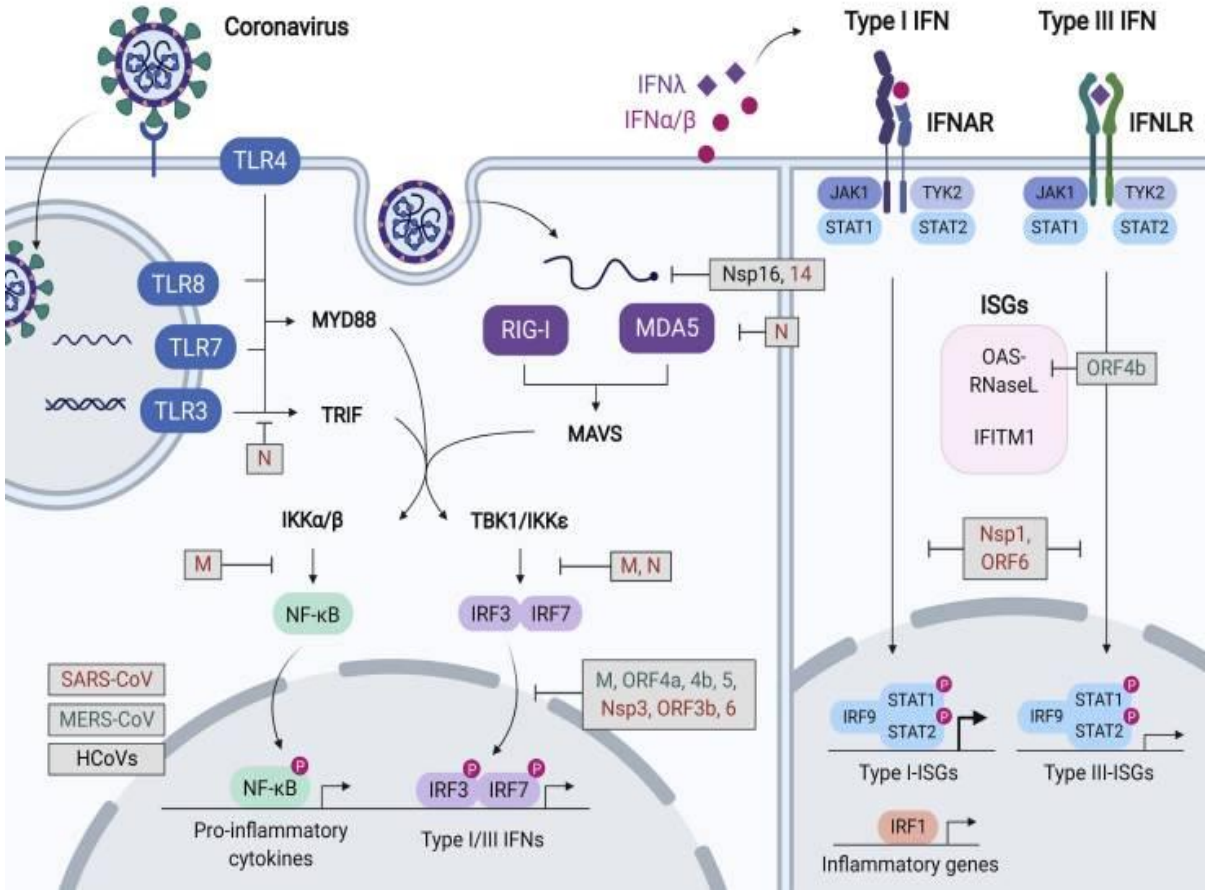
NP structure and self-assembly





Heliyon 6, e04743 (2020)
Cell Host Microbe 27, 325–328 (2020).

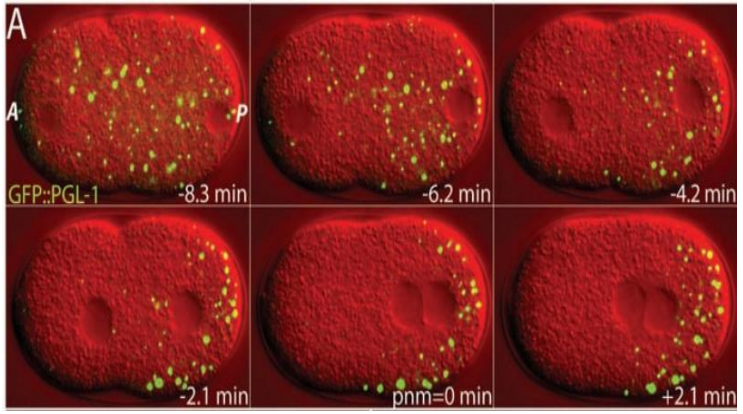
Antiviral infection signaling pathway



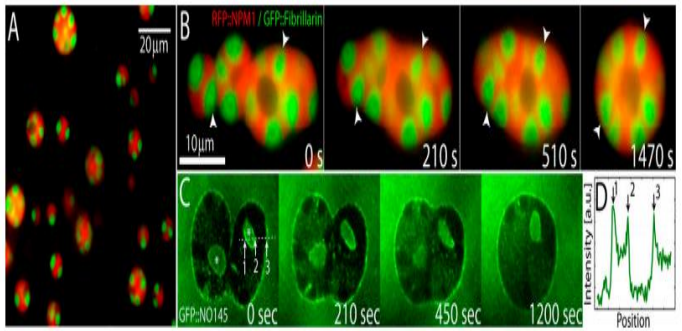
Cell Host & Microbe 27, June 10, 2020

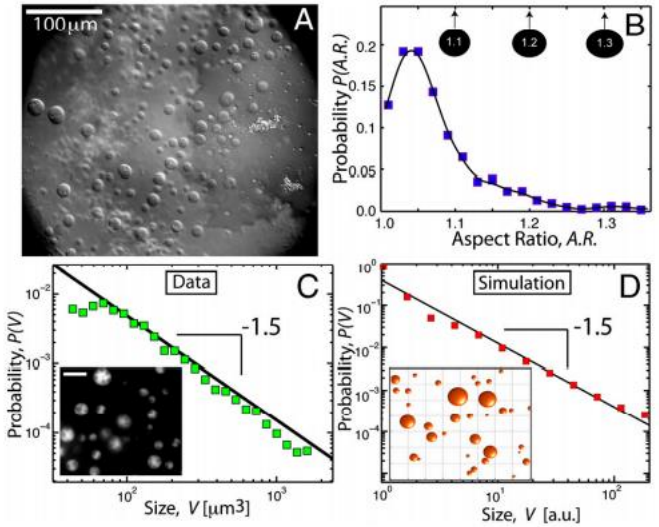
Introduction

Liquid-liquid separation

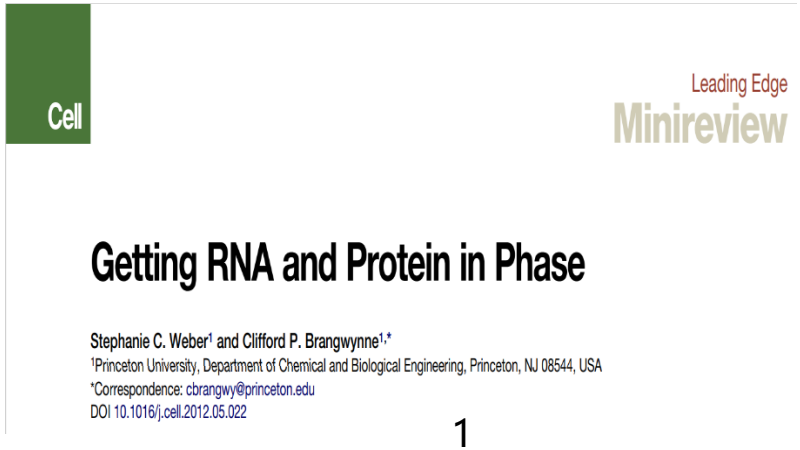


Science 324, 1729 (2009);

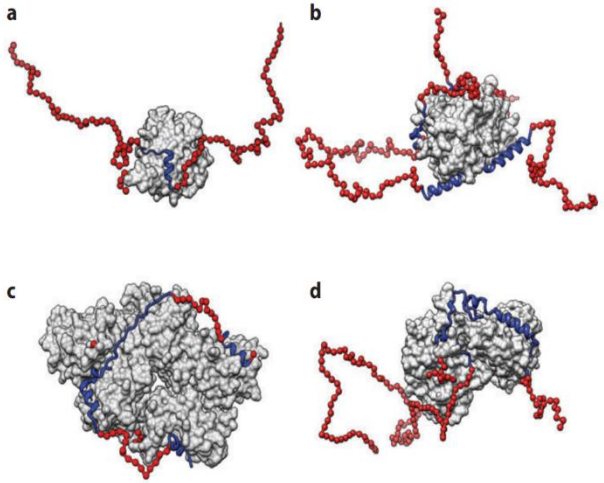




PNAS | March 15, 2011 | vol. 108 | no. 11



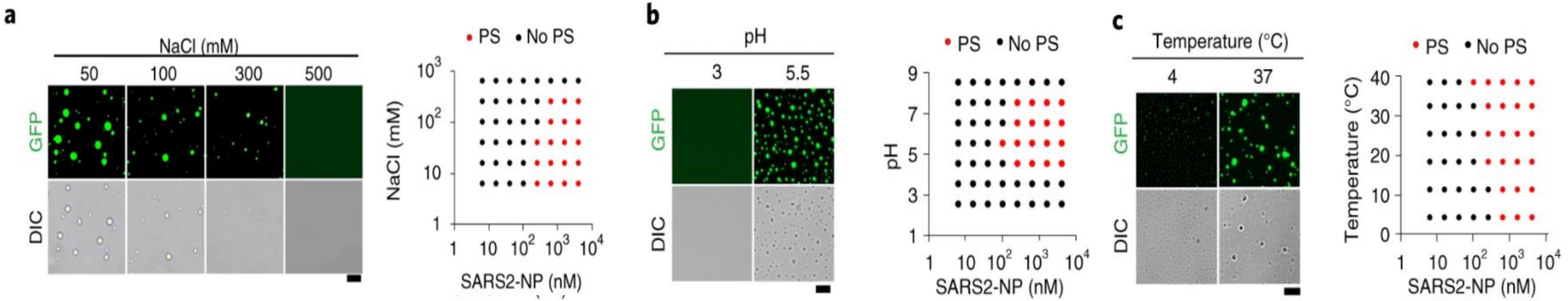
Cell 149, June 8, 2012.



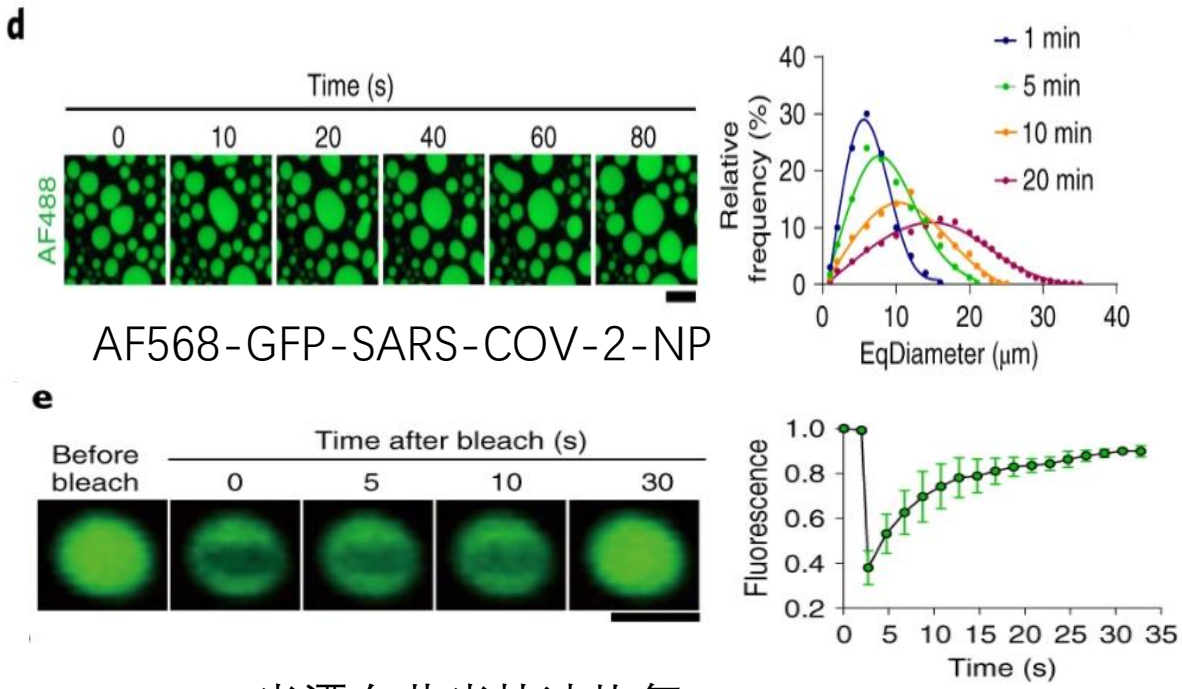
Annu Rev Biochem. 2014; 83:553–584.

检测相分离技术:
FRAP（光漂白荧光快速恢复）
活细胞成像
体外重构纯化蛋白

体外验证NP是否能发生LLPS

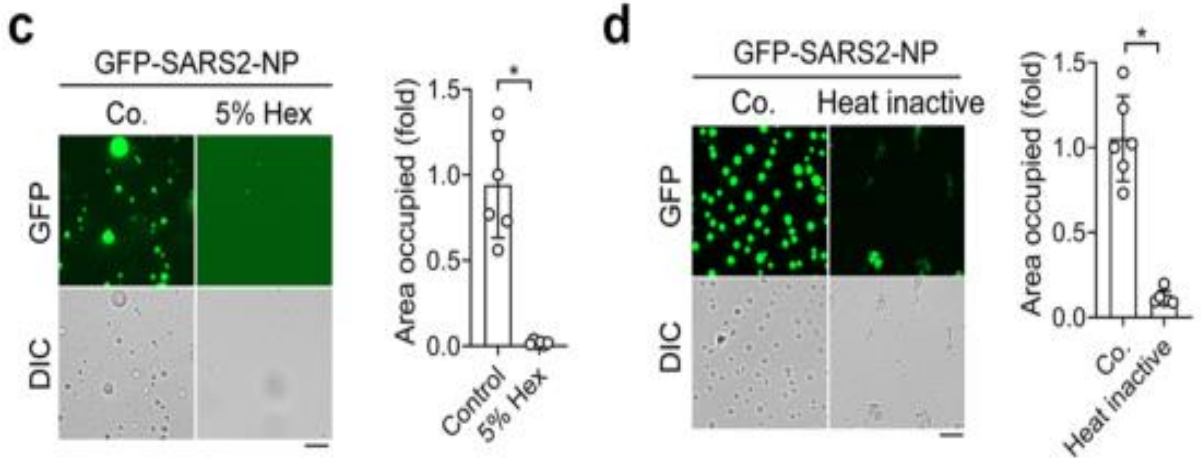


Extend fig1



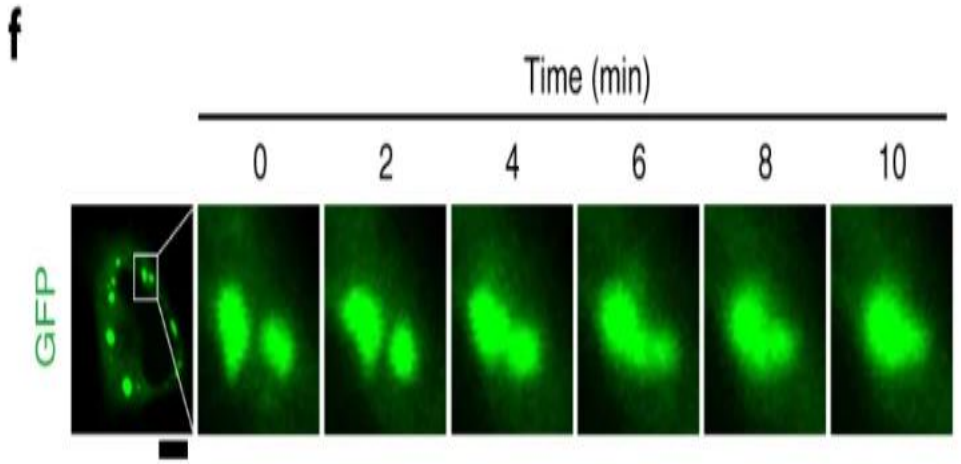
AF568-GFP-SARS-COV-2-NP

FRAP：光漂白荧光快速恢复

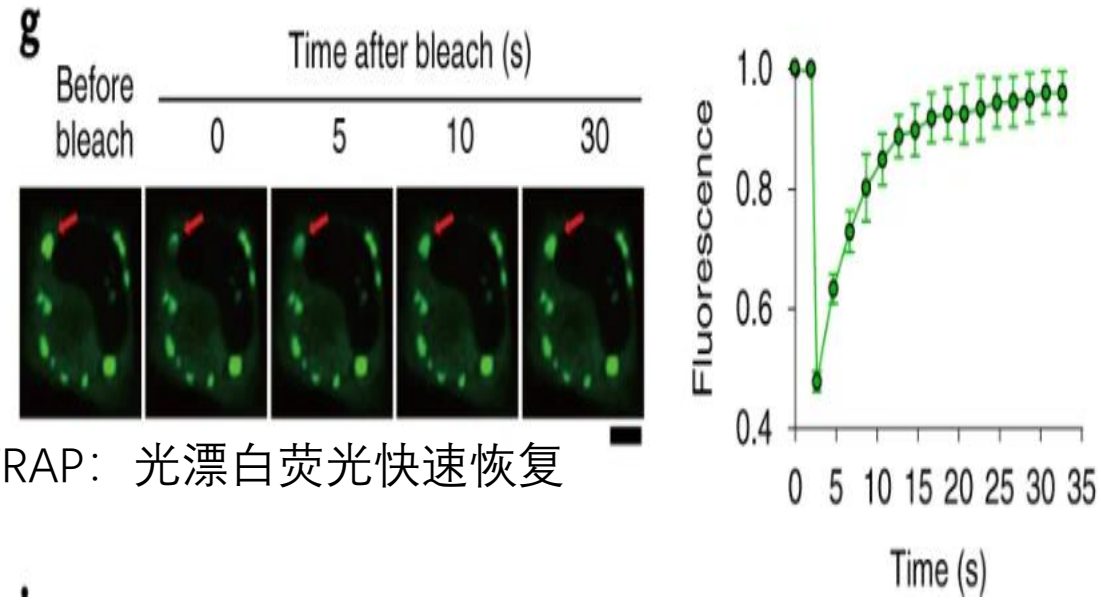


结果：SARS-COV2-NP在体外可以发生液液相分离

胞内验证NP发生是否能发生LLPS

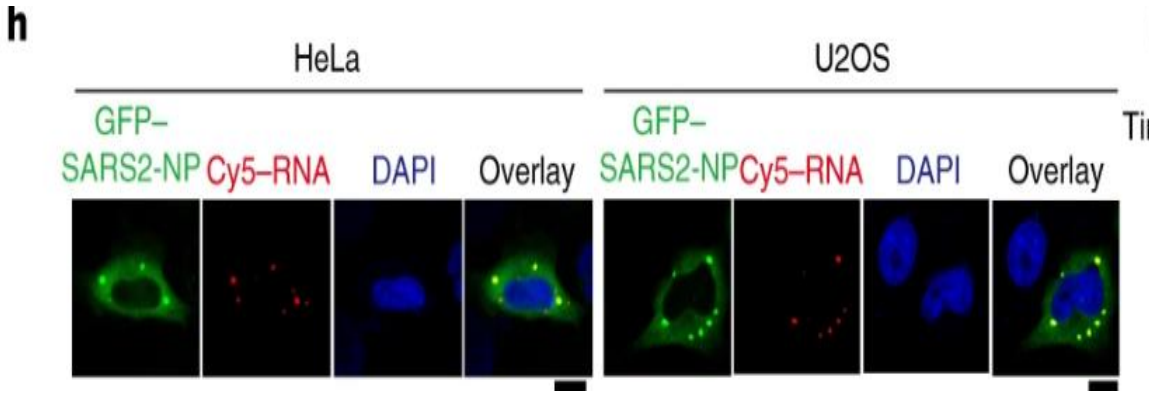


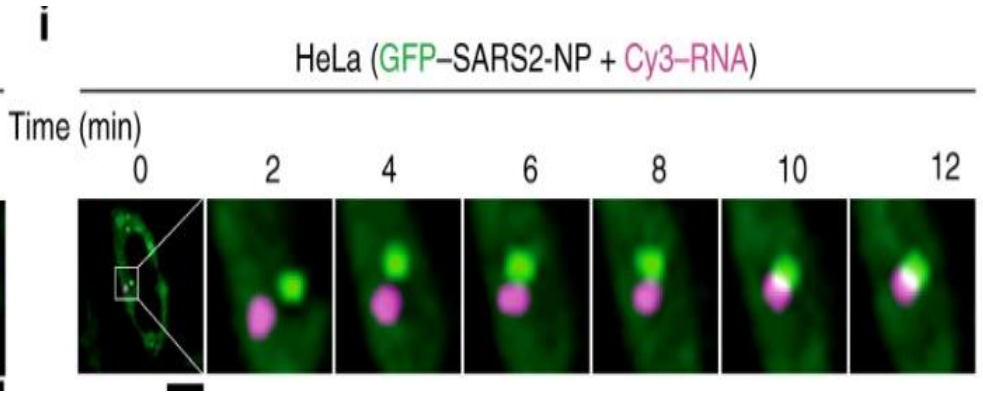
Hela cell



FRAP：光漂白荧光快速恢复

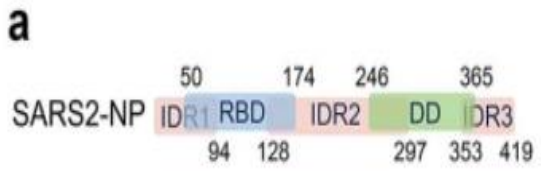
RNA是否参与NP液滴形成

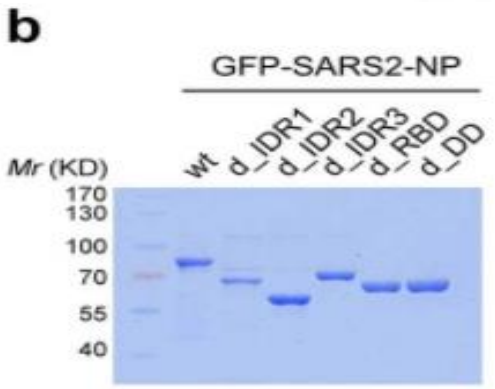


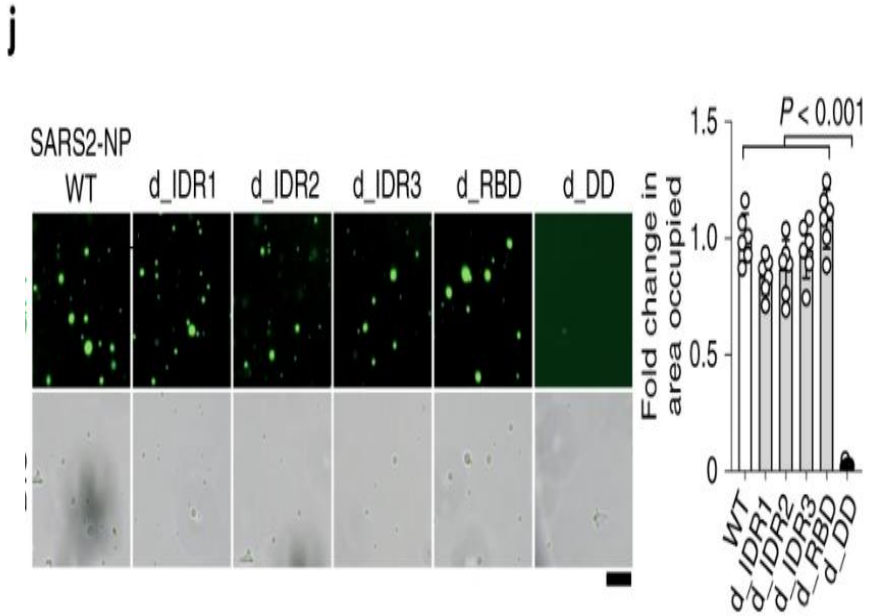


结果：　SARS-COV-2-NP在细胞内能发生液液相分离

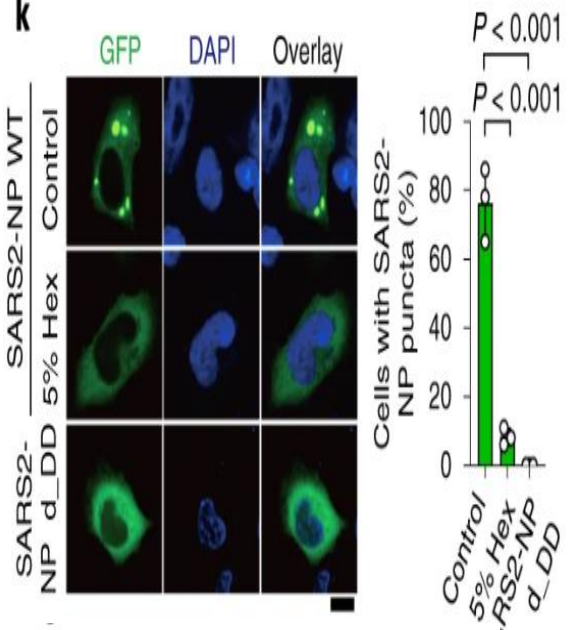
验证NP发生LLPS的区域

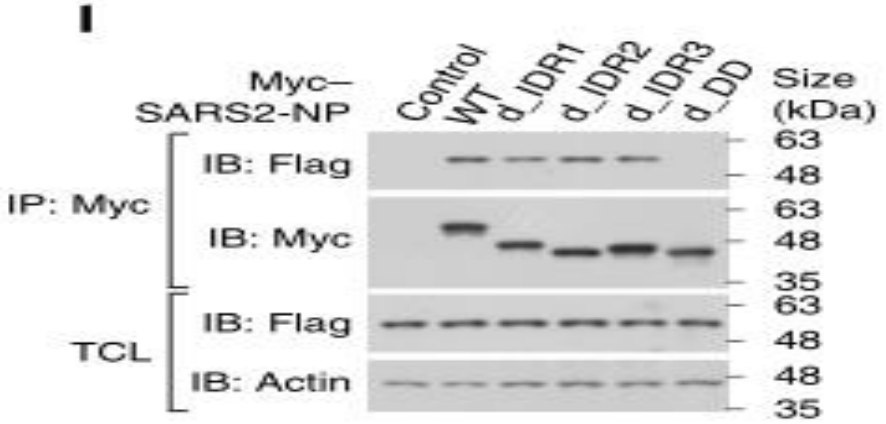




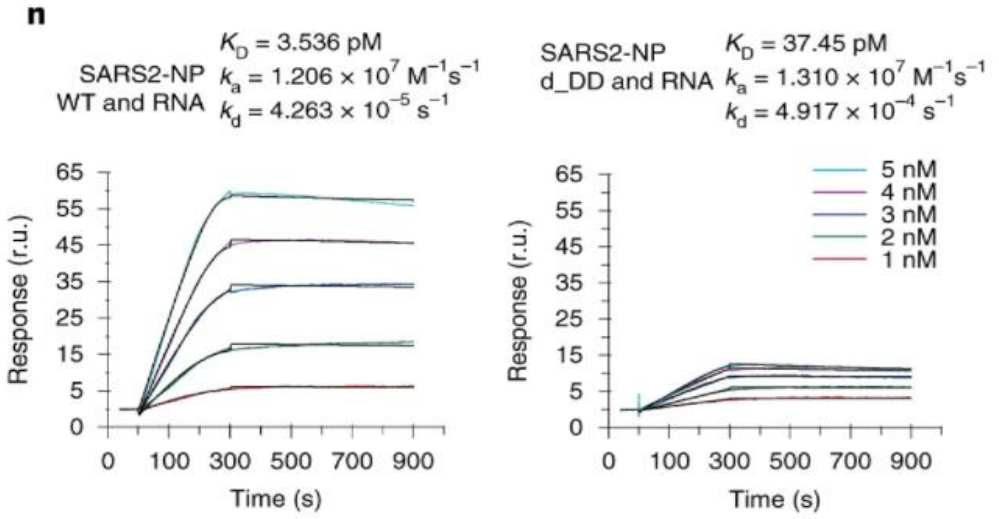


Hela细胞



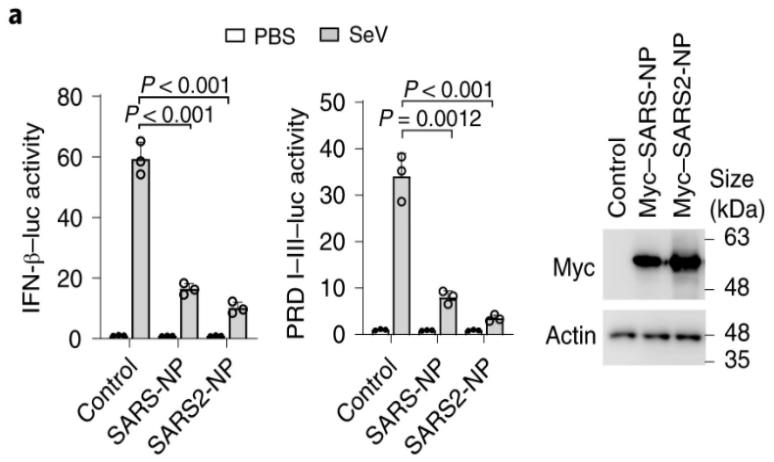


IP:免疫共沉淀

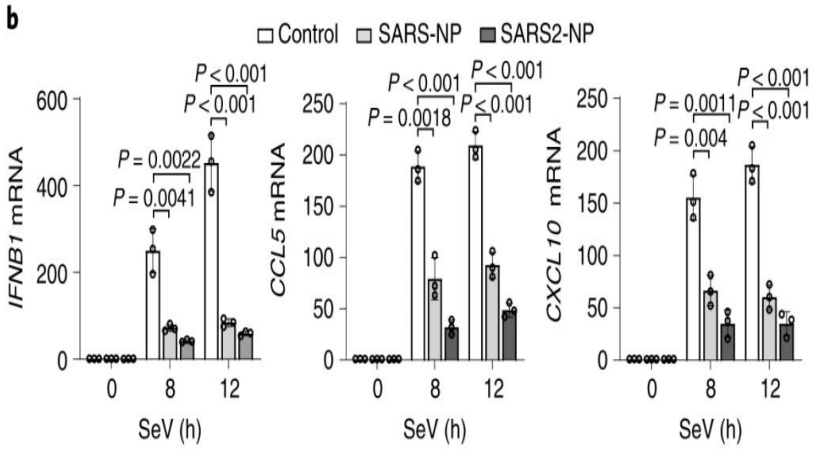


结果:二聚化结构域是NP发生液液相分离的区域

验证SARS-NP在先天抗病毒免疫中的功能



免疫印迹



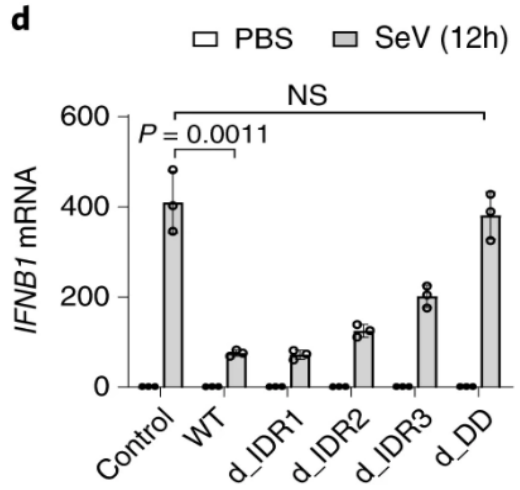
qpcr

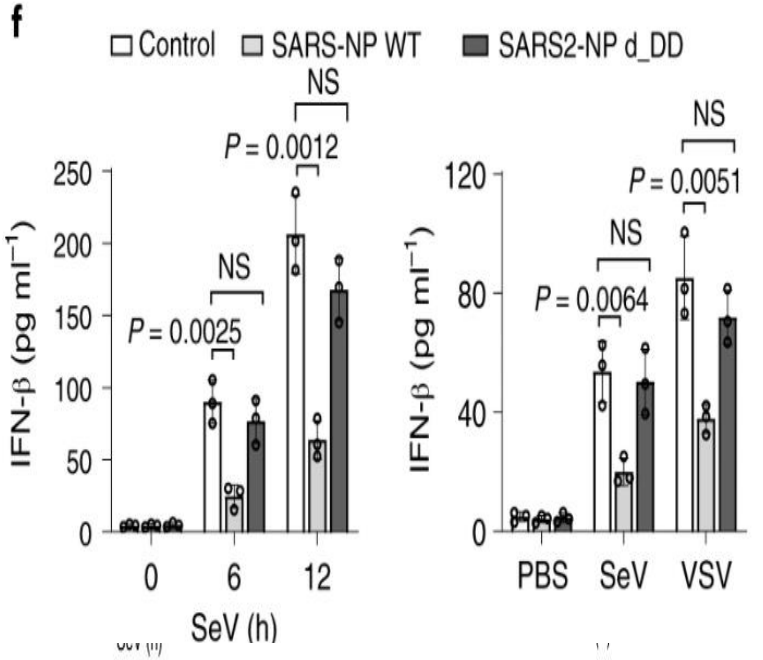
构建编码GFP-SARS-COV-NP和GFP-SARS-COV2-NP、IFN-β 和 PRD I-III（包含 Ifnb1 启动子的 IRF3 结合位点）的报告质粒转染 HEK293T 细胞，Sev病毒刺激

结果：外源性过表达GFP-SARS-COV-NP、SARS-COV2-NP导致干扰素活性及干扰素mRNA表达水平面显著降低

体外验证SARS-NP-DD在先天抗病毒免疫中的功能

qpcr

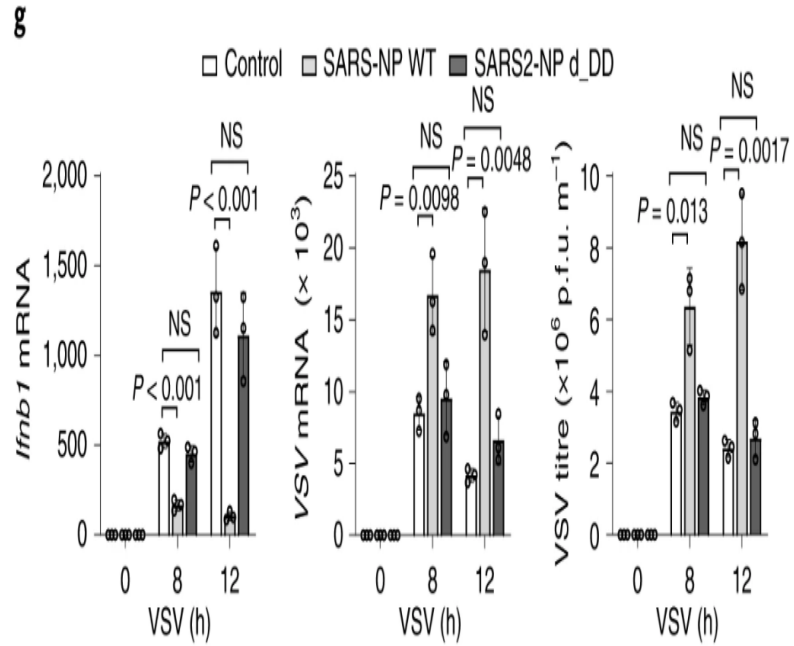


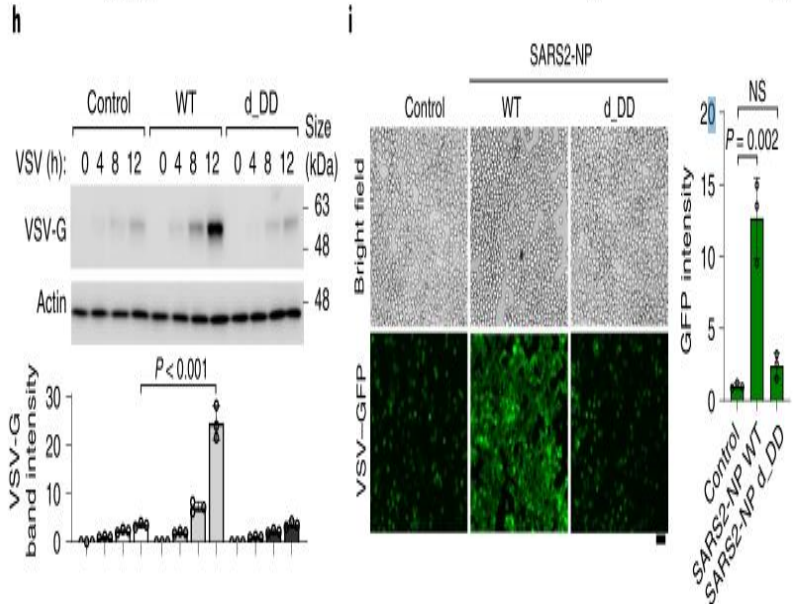


A549cell

ELISA

qpcr

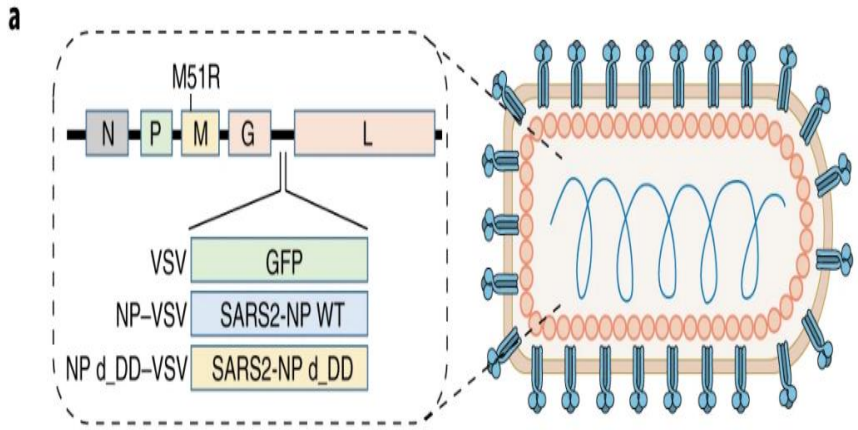


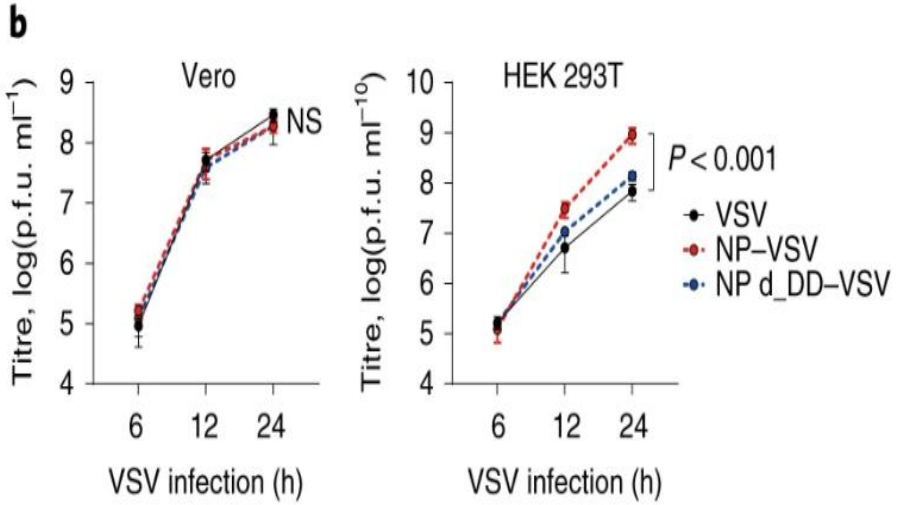


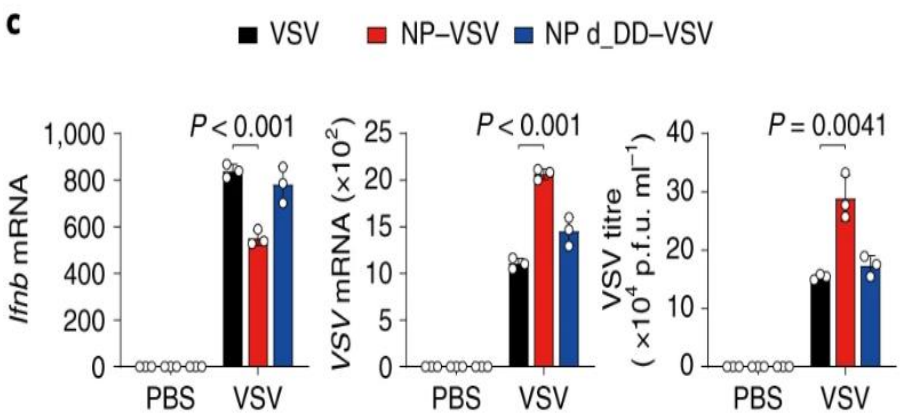
结果:缺失GFP-SARS-COV2-NP 的二聚体结构域dd对干扰素基因的表达没有显著影响

结论：体外SARS2-NP依赖二聚体结构域DD 负向调节 IFN-β 信号传导

体内验证SARS-NP-DD在先天抗病毒免疫中的功能

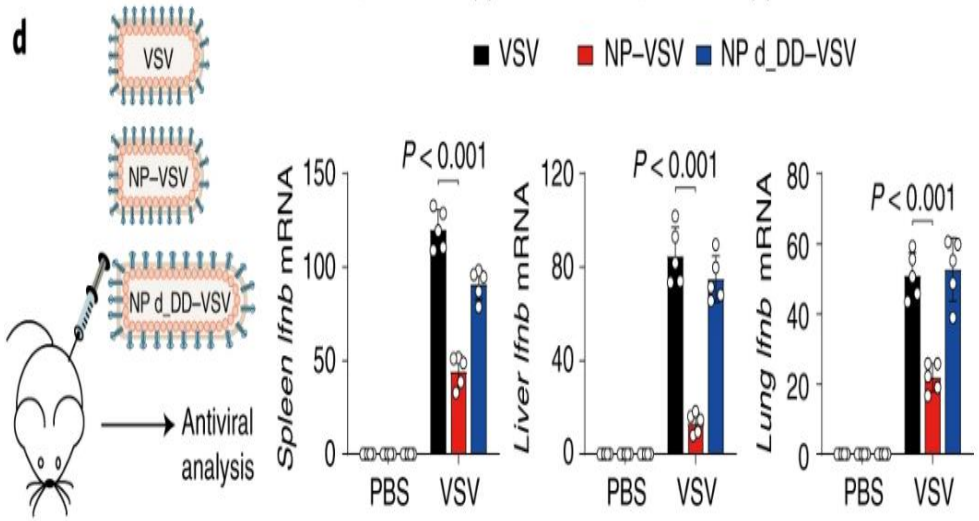


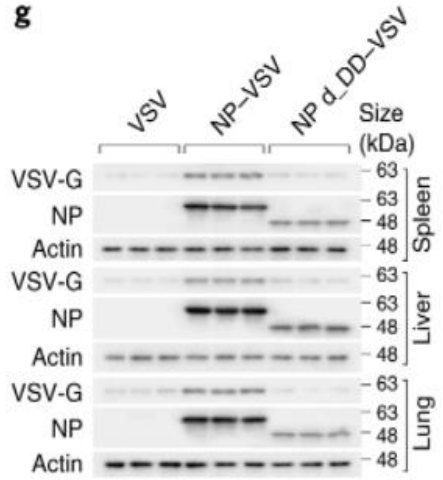




小鼠腹膜巨噬细胞

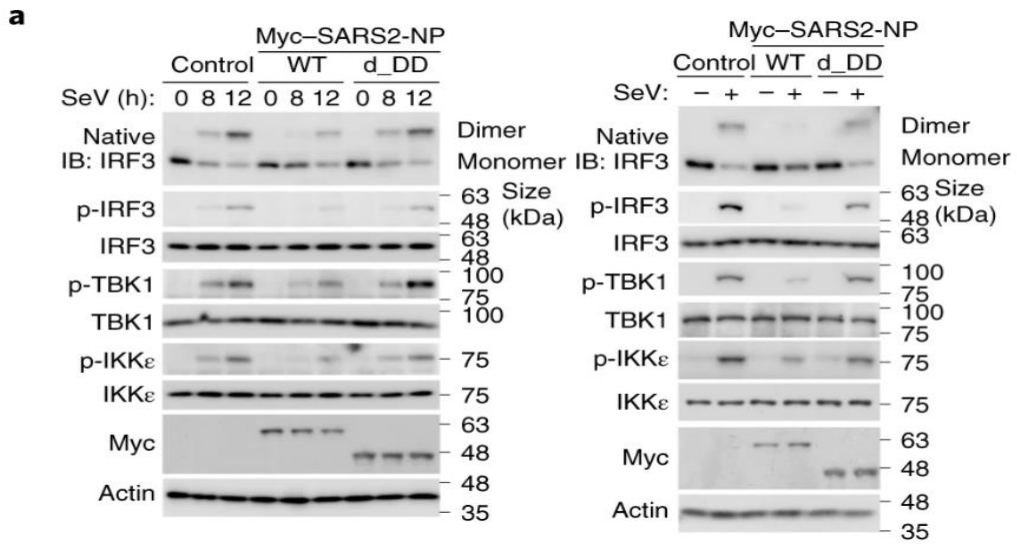
Normalized *Ifnb1* mRNA expression (left) and *VSV* copy number（right）





Normalized *Ifnb1* mRNA expression in the spleen (left), liver (middle) and lungs (right) of mice

SARS2-NP 负向调控MAVS–IRF3激活



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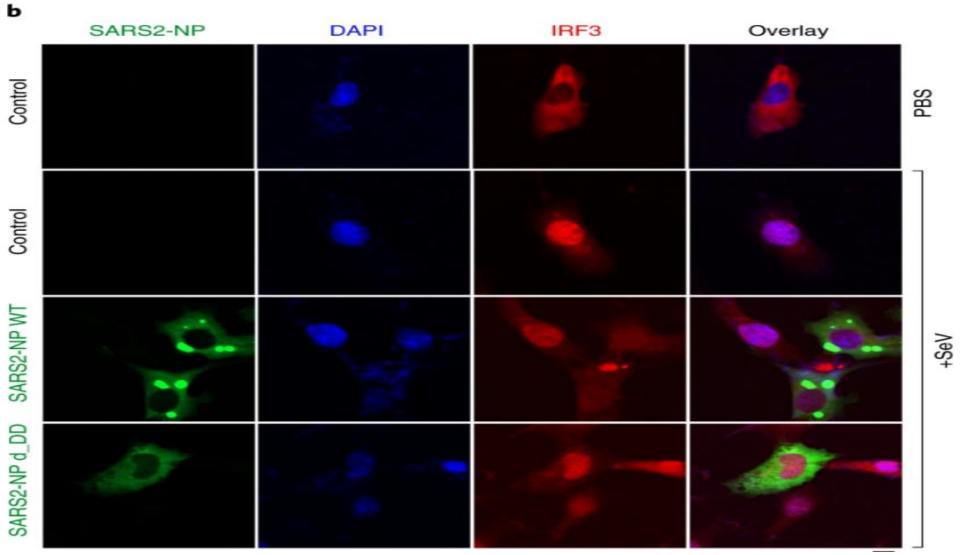

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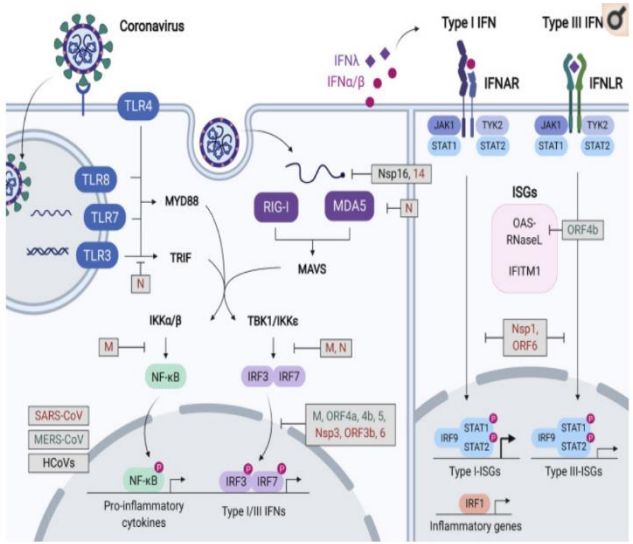
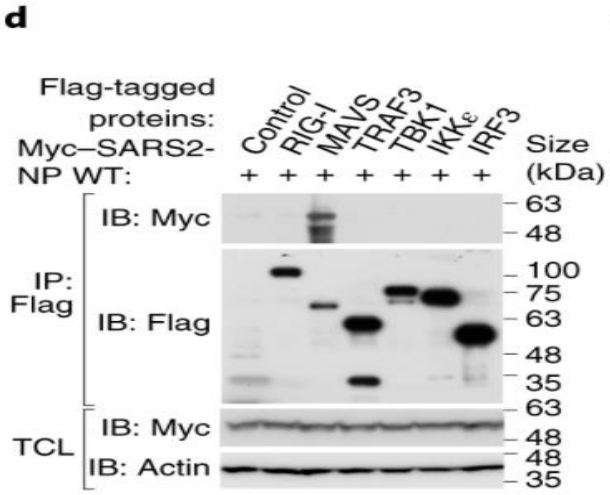
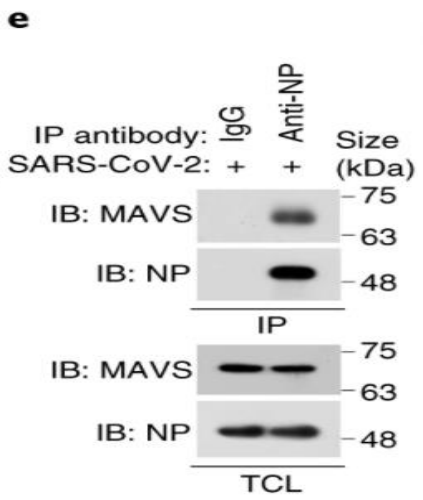
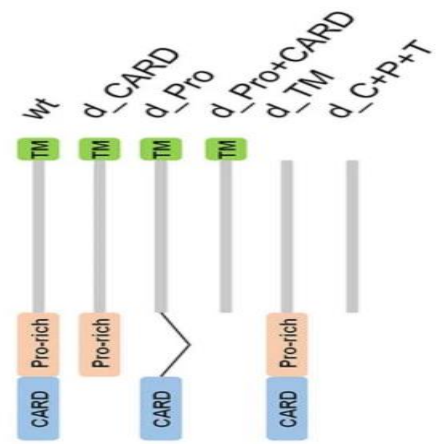


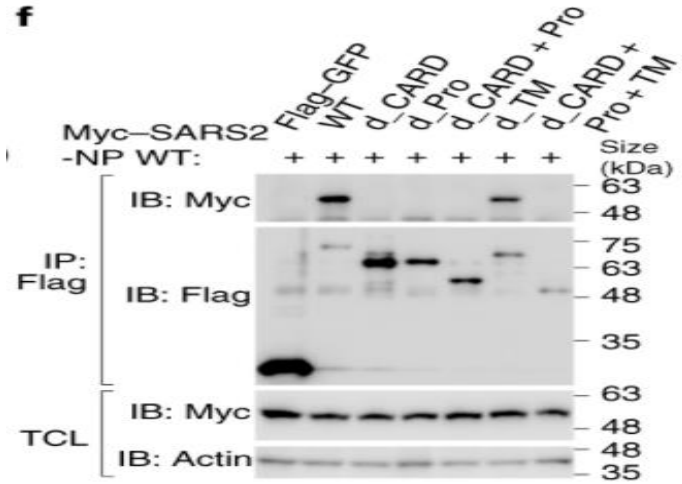




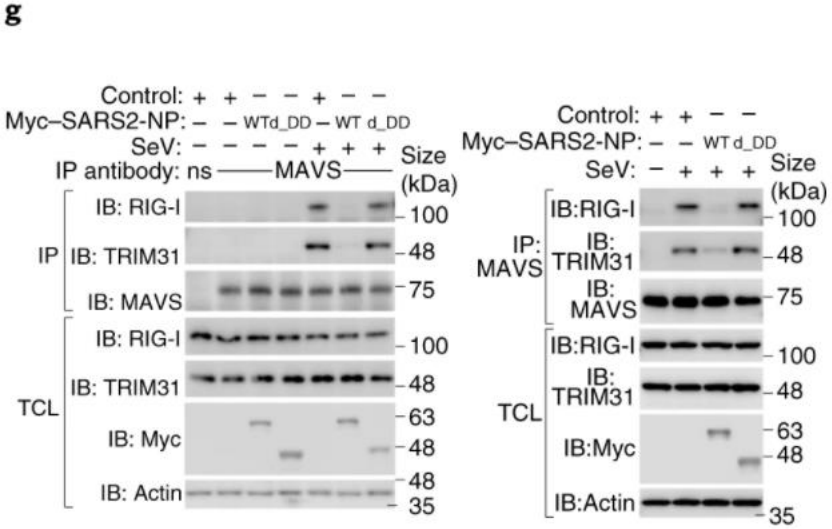
免疫沉淀



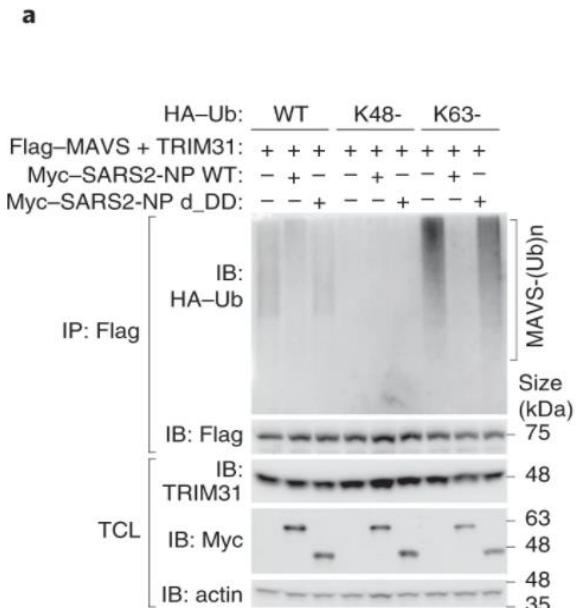
SARS2-NP 通过抑制MAVS泛素化负向调控MAVS–IRF3激活

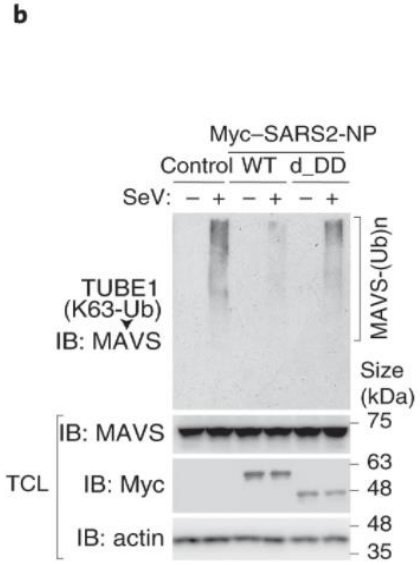


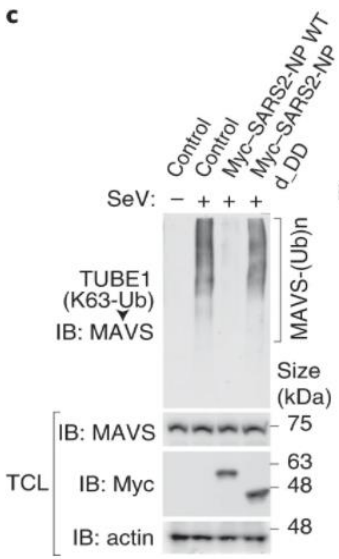
确定 MAVS N末端结构域CARD和pro区与SARS2-NP作用

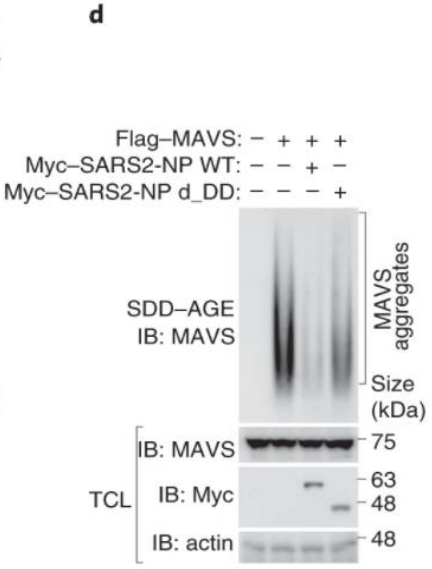


NP抑制MAVS与泛素化连接酶TRIM31的作用

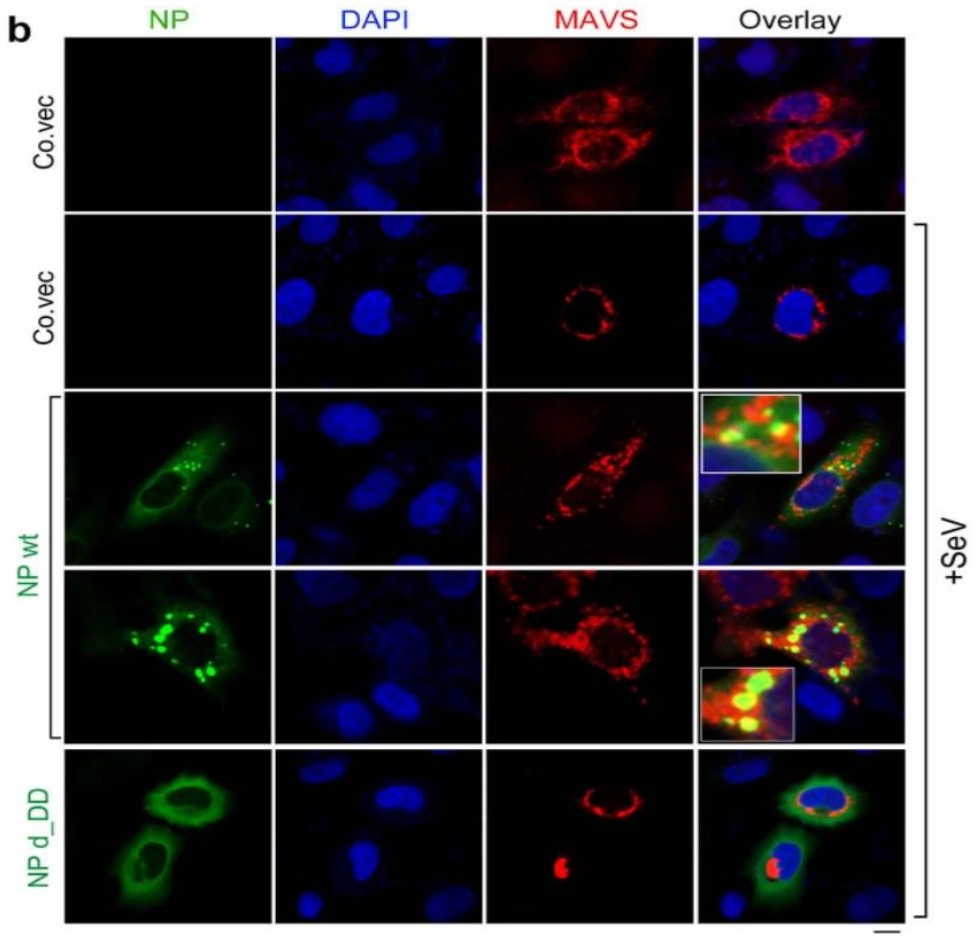




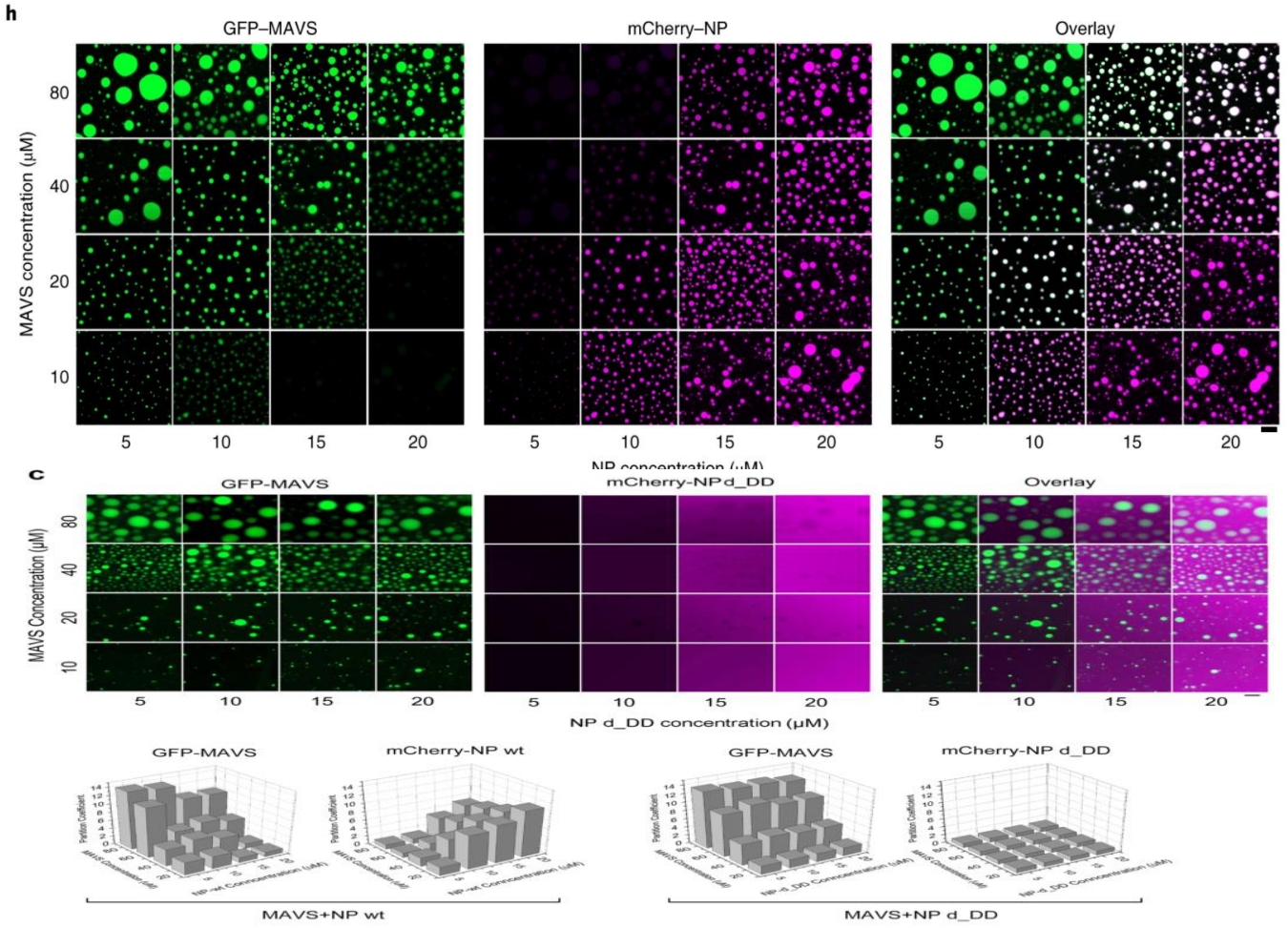




SARS2-NP 通过抑制MAVS泛素化负向调控MAVS–IRF3激活

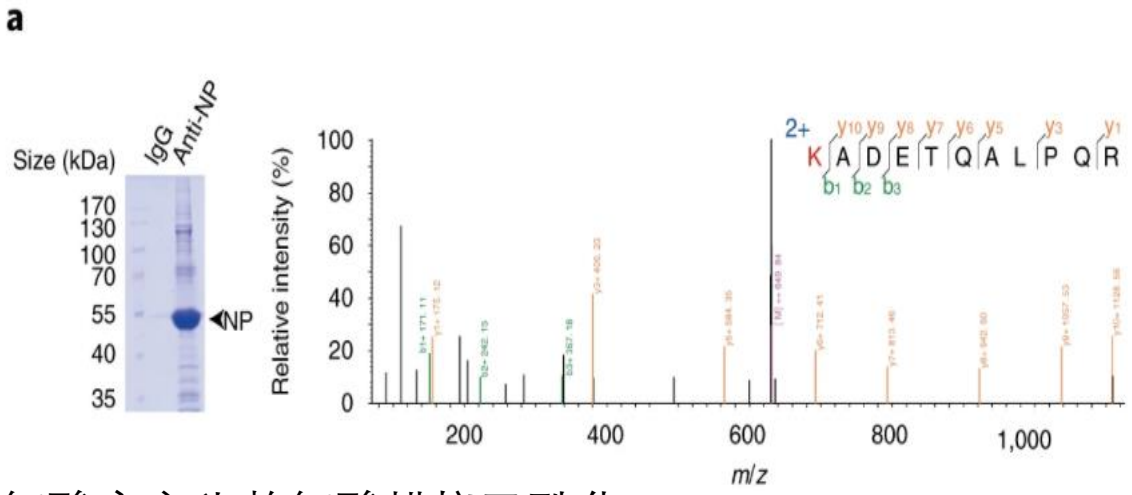


MAVS被SARS-NP破坏成段，不会被SARS-NP-d-DD破坏

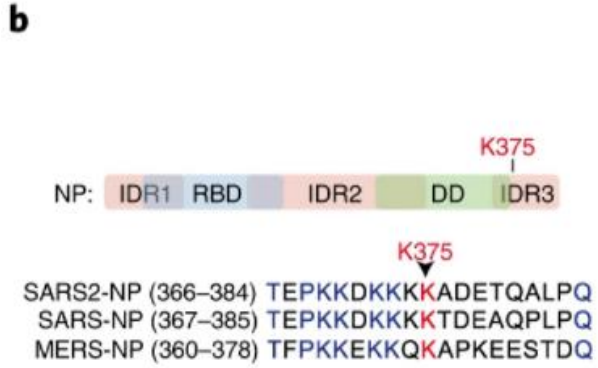


SARS-NP相分离干扰MAVS相分离

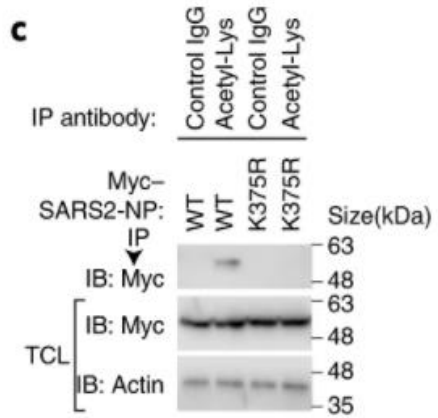
SARS2-NP 在Lys375位点的乙酰化消除了 NP介导的LLPS和MAVS信号通路抑制

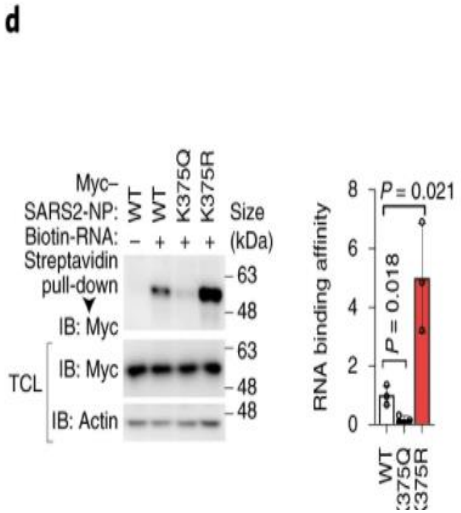


谷氨酸突变为赖氨酸模拟乙酰化

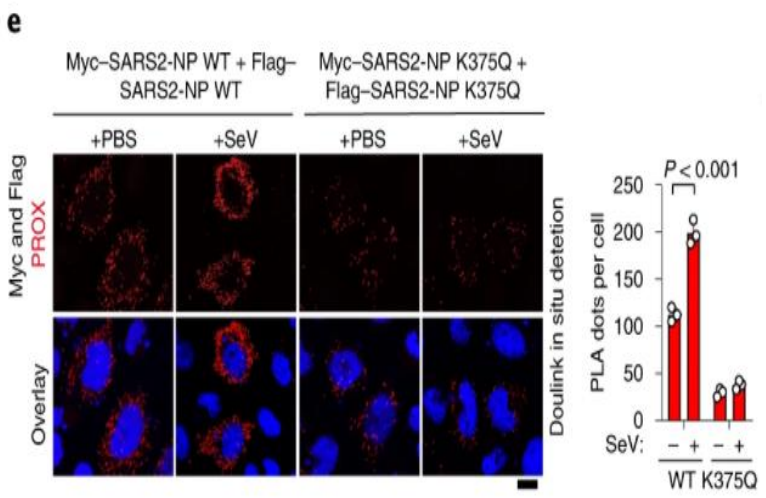


K375Q位点乙酰化

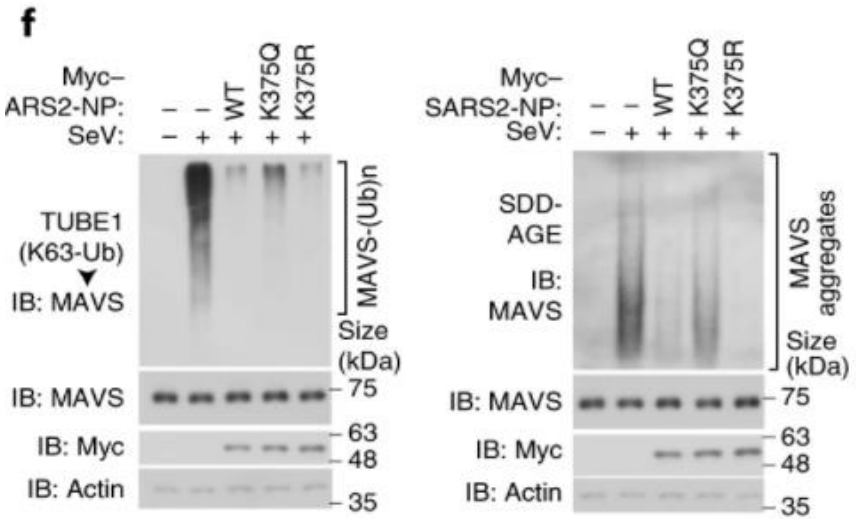




NP与RNA结合效率分析



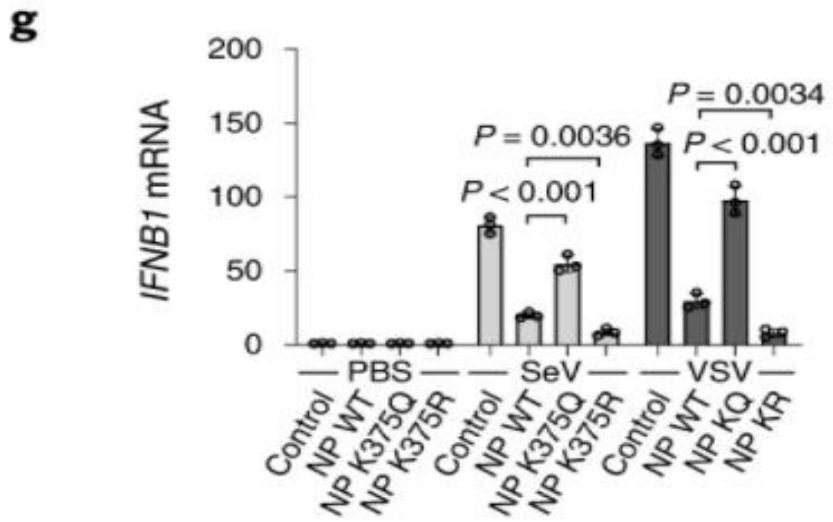
K375Q对病毒的诱导能力明显降低



K375Q对MAVS泛素化抑制减弱

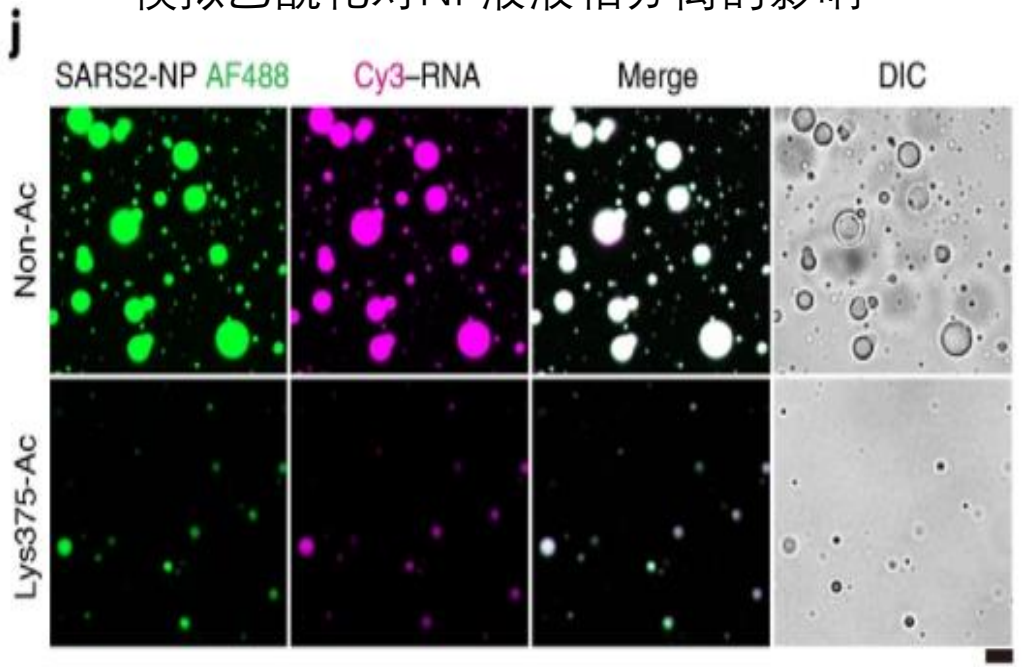
SARS2-NP 在L375位点的乙酰化消除了 NP介导的LLPS和MAVS信号通路抑制

模拟乙酰化对干扰素表达的影响



A549cell共转染质粒,Sev和VSV病毒刺激12h

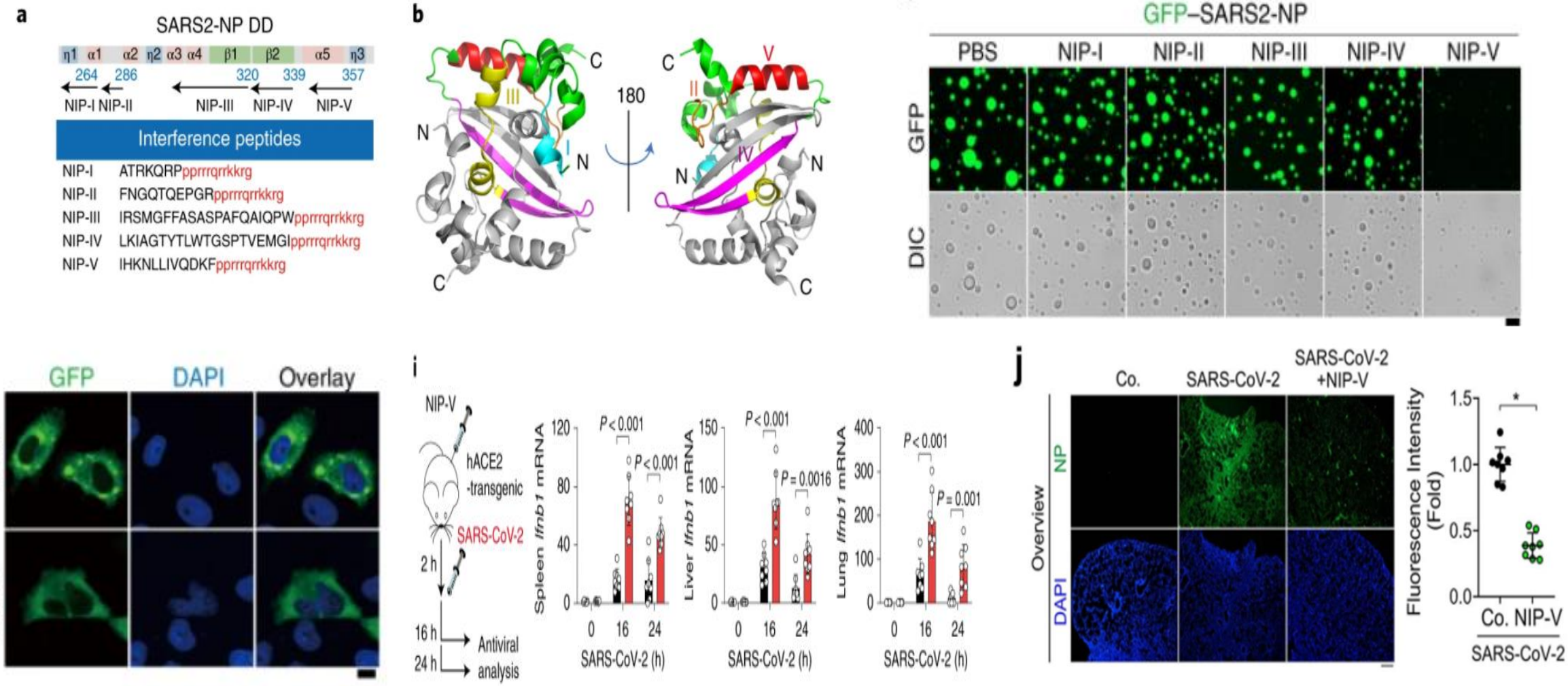
模拟乙酰化对NP液液相分离的影响



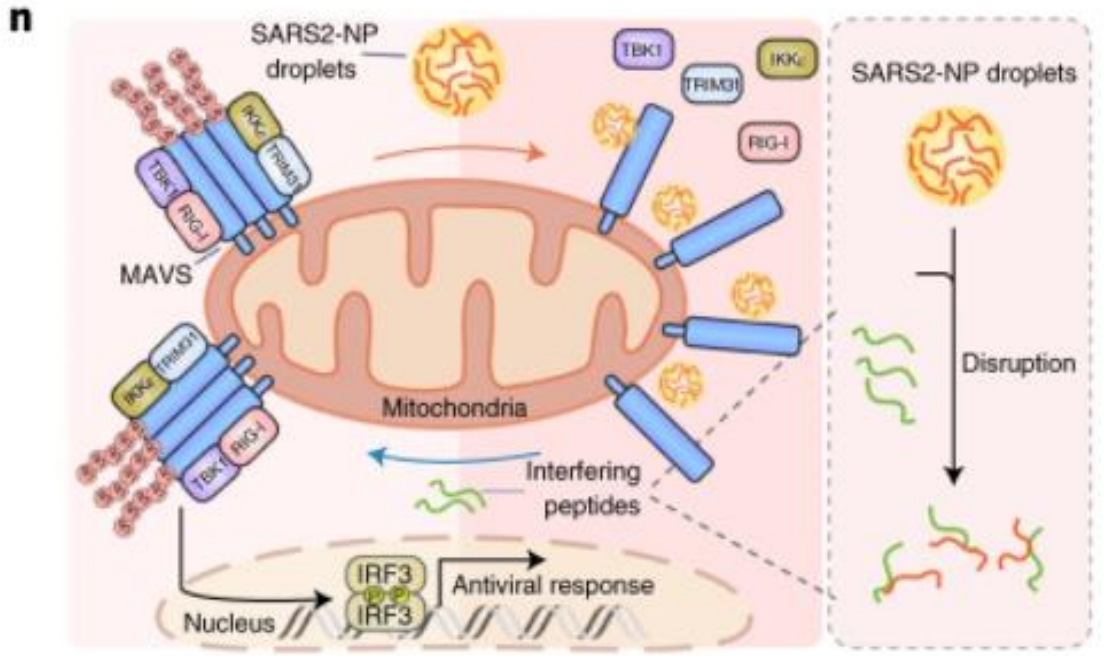
AF488 标记的 SARS2-NP WT（非 A乙酰化）与cy3-RNA混合液滴形成
SARS2-NPLys375（乙酰化）（5 μM）与Cy3-RNA（5 μM）混合的液滴形成

结论：Lys375 处的 NP 乙酰化消除了NP的 LLPS，部分恢复MAVS泛素化，提高了MAVS活性

Interfering peptide NIP-V targeting the DD disrupts SARS2-NP LLPS and thus enhances the innate antiviral response both in vitro and in vivo.



summary



总结：文章阐明了SARS-CoV-2 NP作用于先天免疫信号通路MAVS的机制，提出了干扰其液液相分离，提升宿主先天抗病毒免疫的可行性，并进一步针对性地设计了有效的抑制病毒复制，增强宿主先天抗病毒免疫的干扰肽。

- SARS-NP是否能发生相分离
- SARS-NP相分离发生的区域
- 发生相分离的NP是否介导先天抗病毒免疫逃逸
- 发生相分离的NP通过抑制MAVS泛素化负向调控抗病毒感染信号通路
- SARS-NP的lys375乙酰化抑制相分离，增强MAVS活性
- 设计靶向SARS-NP-DD的干扰肽，增强抗病毒免疫