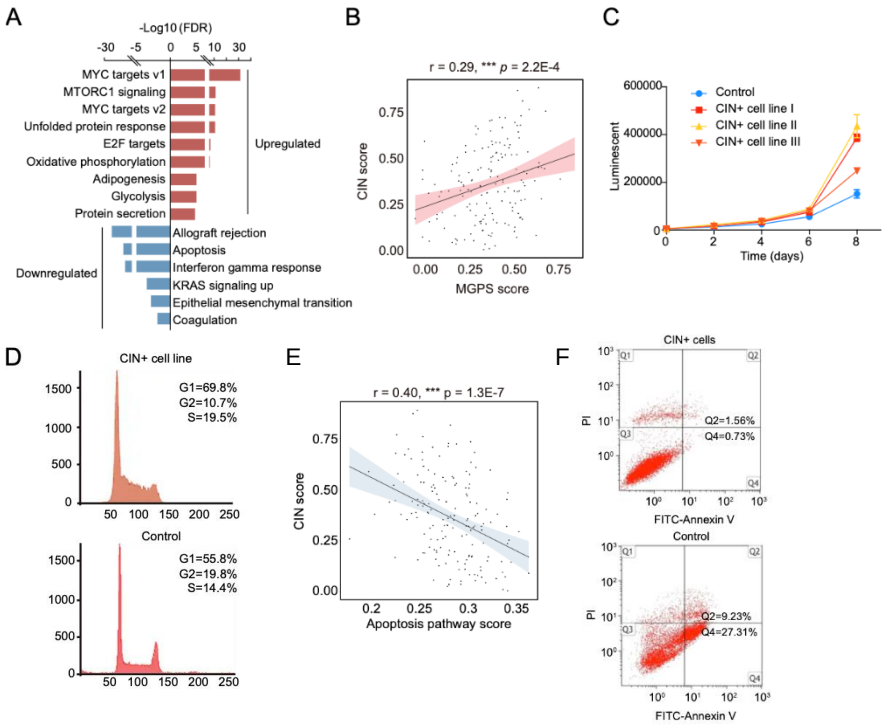
超速流式细胞分选仪在颅底脊索瘤多组学研究中的应用

复旦大学丁琛教授课题组于*Nature Communications*杂志上发表题为“**Proteogenomic characterization of skull-base chordoma**”的文章 (Q. Zhang, et al. C. Ding, Nat Commun. 2024 Sep 27; 15(1):8338. doi: 10.1038/s41467-024-52285-7)。在研究过程中，丁琛教授课题组利用国重平台的**超速流式细胞分选仪BD FACS Calibur**和**MoFlo-XDP**测定CIN（染色体不稳定性）+细胞中的细胞增殖与细胞凋亡水平，以确定CIN对颅底脊索瘤肿瘤细胞增殖和凋亡的调控。最后结果验证了我们的假设，在颅底脊索瘤中，高的染色体不稳定性促进了肿瘤细胞增殖，同时抑制了细胞凋亡。



A. CIN+组和CIN-组的蛋白质组特征通路。
B-D. CIN score与肿瘤细胞增殖呈正相关。
E-F. CIN score与肿瘤细胞凋亡呈负相关。

Gene set enrichment analysis (GSEA) of differentially expressed proteins (DEP) based on proteomic data showed that oncogenic pathways, including MYC targets, E2F targets, mTORC1 signaling, and DNA repair pathways, were significantly upregulated in CIN+ skull base chordomas. And there was a significant positive correlation between CIN score and tumor proliferation score. Therefore, the author generated monoclonal CIN+ cells through Cytabirine treatment, and further measured the proliferation level of CIN+ cells using a high-speed flow cytometer, which showed a significant increase. At the same time, GSEA and ssGSEA analysis showed that the apoptotic pathway was downregulated in CIN+ skull base chordomas. Therefore, the author further used a high-speed flow cytometer to determine and found that the apoptosis level of CIN+ cells was significantly reduced.

These results validated the hypothesis that high chromosomal instability promotes tumor cell proliferation and inhibits cell apoptosis in skull base chordomas.

BD FACS Calibur
流式细胞分析仪
负责老师：沈素芹

MoFlo-XDP
超速流式细胞分选仪
负责老师：刘龙江

基于蛋白质组数据的差异表达蛋白质（DEP）的基因集富集分析（GSEA）显示，包括MYC靶标、E2F靶标、mTORC1信号和DNA修复途径在内的致癌途径在CIN+的颅底脊索瘤中显著上调（图A）。且CIN评分与肿瘤增殖评分呈显著正相关（图B）。因此，作者通过Cytabirine处理生成单克隆CIN+细胞，进一步利用超速流式细胞分选仪**BD FACS Calibur**和**MoFlo-XDP**测定，发现CIN+细胞的增殖水平明显增加（图D）。同时GSEA和ssGSEA分析表明，CIN+颅底脊索瘤中细胞凋亡途径的下调（图E）。因此，作者进一步利用超速流式细胞分选仪测定，发现CIN+细胞的凋亡水平明显降低（图F） 。这些结果验证了假设，即在颅底脊索瘤中，高的染色体不稳定性促进了肿瘤细胞增殖，同时抑制了细胞凋亡。

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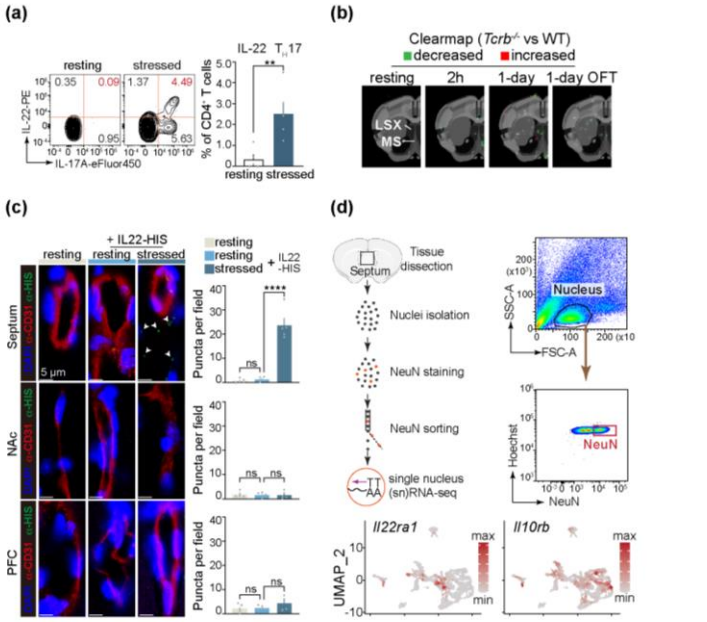
神经细胞单细胞核分选与全脑iDISCO成像

复旦大学于肖飞教授课题组于*Immunity*杂志上发表题为“**Elevated IL-22 as a result of stress-induced gut leakage suppresses septal neuron activation to ameliorate anxiety-like behavior**”的文章（Xia M, Lu J, Lan J et al. Yu X, *Immunity*)。在研究过程中，于教授课题组（1）利用国重平台的流式细胞分析仪Fortessa，检测了压力束缚下小鼠肠道里产生IL-22的细胞类群变化，为IL-22的溯源提供了可靠信息；利用LaVision Ultramicroscope II显微镜 对小鼠全脑神经元激活进行iDISCO成像，为寻找压力束缚下IL-22的调控脑区提供了精准信息；（2）利用国重平台的尼康A1正置激光扫描共聚焦显微镜，检测到压力束缚下IL-22渗入脑隔区（Septum），为明确IL-22是否直接调控Septum提供可靠证据；（3）利用国重平台的BD FACSAria cell sorter流式细胞分选仪，对小鼠Septum区域的神经细胞单细胞核进行分选，为确定Septum是否表达IL-22受体IL22ra1和IL10rb提供最直接的证据。



图a. 流式细胞分析鉴定IL-22$^+$$T_H$17细胞类群；图b. iDISCO显示Septum区域神经元激活情况；图c. 压力束缚下IL-22对不同脑区的渗入情况展示;图d. 神经细胞单细胞核分选及IL-22受体表达分析。

Stress causes immune activation and excess immune activation often becomes pathological. However, our study revealed that stress-induced immune activation also provided adaptive benefits. Taking the early stage of immune activation as the time node, we studied that IL-22 produced by intestinal T_H17 cells entered septal area of the brain via the damaged blood-brain barrier and ameliorated stress induced anxiety by directly inhibiting neuronal activation. This study not only uncovered another physiological function of IL-22, but also revealed the active role of the immune system in defending against psychological damage.

肠道内的IL-22主要来源于T_H17 cell、γδT cell 和ILC3 cell，由于肠道内免疫细胞类型复杂且不同类型细胞占比差异很大，想要利用流式细胞分析的方法从中区分出这三类细胞需要多色抗体同时标识且要求高速高效。而流式细胞分析仪Fortessa拥有4个激光器，可同时检测18种颜色，具有高效、高灵敏度、高分辨率等优点，能够较好的满足我们的要求。

利用常规的IHC方法对全脑的神经元激活进行cFOS染色不仅考验切片技术而且耗时、误差较大，而利用iDISCO和LaVision Ultramicroscope II 显微镜成像技术不仅能克服上述缺点，且能够更加直观、清晰、准确的判断出不同脑区的神经元激活情况，这为寻找IL-22可能的调控脑区提供了精准信息。

大脑的微血管结构和渗入Septum区域的小分子IL22-HIS需要高分辨率显微镜才能被清晰观察，而国重平台的尼康A1显微镜能够满足我们对高分辨率、高清晰度的要求。

由于神经细胞难分离、分离后体外存活率低、存活时间短等特点，较难实现神经细胞分选后的单细胞测序。在该研究中，我们对神经细胞的单细胞核进行提取，后利用BD FACSAria cell sorter流式细胞分选仪对NeuN$^+$单细胞核（神经细胞核）分选后进行单细胞核测序，该分选仪器具有高分辨率、高流速、高分选效率等特点，能够在较短的时间内实现高质量分选。

BD FACS Calibur
流式细胞分析仪
负责老师：沈素芹

Nikon A1
正置激光扫描共聚焦显微镜
负责老师：杨建鸽

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超高分辨成像观察OLFM4⁺ MV结合受体细胞FAT1蛋白

复旦大学周兆才教授课题组在*Nature Communications*杂志上发表题为“**Epstein-Barr virus infection upregulates extracellular OLFM4 to activate YAP signaling during gastric cancer progression**”的文章(F. W. et al., *Nat Commun.*, 2024, 15(1):10543)。在研究过程中，周教授课题组利用国重平台的超分辨共聚焦显微镜观察到EBV阳性胃癌病人肿瘤组织样本来源的OLFM4⁺胞外囊泡能够结合到受体细胞的胞外。进一步地超高分辨观察发现OLFM4⁺胞外囊泡与受体细胞膜上的FAT1蛋白共定位，可参与调控受体细胞胞内Hippo/YAP信号通路，进而影响受体细胞的活动。

Zeiss LSM880 with Airyscan
超分辨共聚焦显微镜

Zeiss LSM710
高感度激光扫描共聚焦显微镜

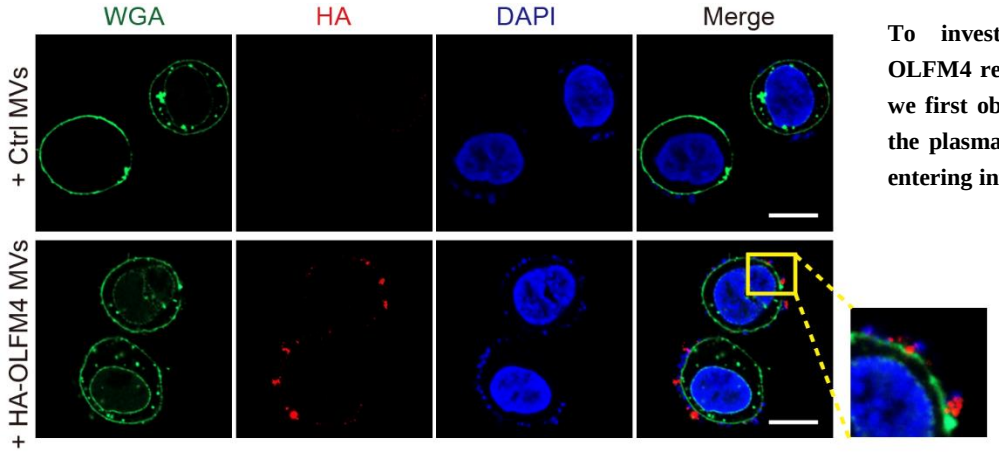

图1 超分辨共聚焦观察分泌到胞外的OLFM4+MV在受体细胞的定位

To investigate the molecular mechanism by which OLFM4 regulates Hippo/YAP pathway in recipient cells, we first observe MV-delivered OLFM4 was localized on the plasma membrane of the recipient cells (Fig. 1), not entering into recipient cells.

Considering that OLFM4 can bind pan-cadherin and that FAT1 is a nonclassical cadherin protein which previously identified as a key upstream regulator of the Hippo pathway. Ultra-high resolution imaging reveals MV-delivered HA-tagged OLFM4 was well colocalized with FAT1 on recipient cells (Fig. 2).

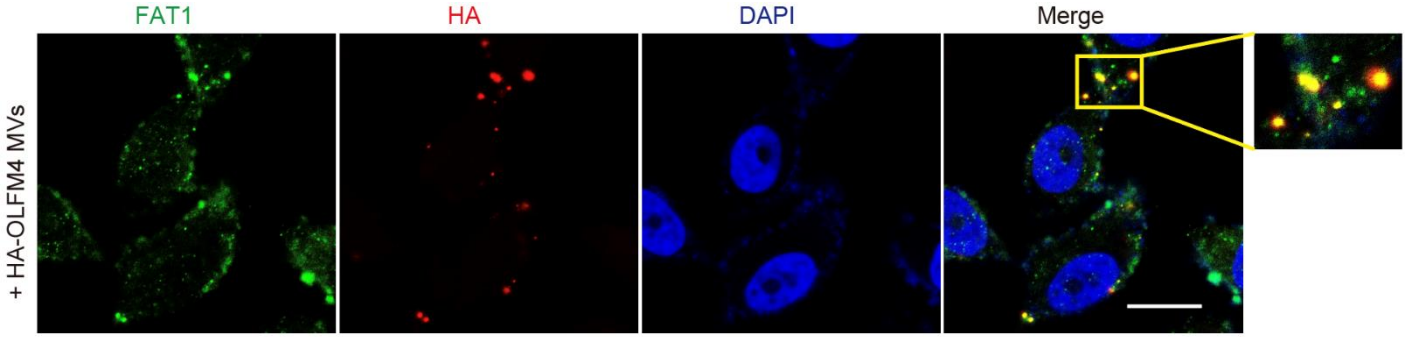

图2 超分辨共聚焦观察分泌到胞外的OLFM4+MV与受体细胞膜上的FAT1蛋白共定位

传统的荧光成像显微镜在观察细胞亚细胞器结构时，由于分辨率不足，难以清晰地区分亚细胞结构，在本研究中采用了国重平台横向分辨率为120 nm的Zeiss-LSM880 with Airyscan超分辨共聚焦显微镜，Airyscan接受相当于传统针孔1.25倍的光线，大幅改善影像的信噪比，清晰地观察到细胞的亚细胞器-细胞膜以及微囊泡MV的定位。

为了探究OLFM4⁺ MV在受体细胞中调控Hippo/YAP信号通路的分子机制。我们利用国重平台的Zeiss-LSM880 with Airyscan超分辨共聚焦显微镜发现HA标签OLFM4⁺ MV定位在受体细胞外膜上。进一步地利用国重平台的Zeiss-LSM880 with Airyscan超分辨共聚焦显微镜，清晰地观察到OLFM4⁺ MV与受体细胞膜上的FAT1共定位，且在细胞膜上聚集成点状。

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复旦大学于王久存教授课题组于*Journal of Advanced Research*杂志上发表题为“**miR-3606-3p alleviates skin fibrosis by integratively suppressing the integrin/FAK, p-AKT/p-ERK, and TGF-β signaling cascades**”的文章（Chen Y, et al. *Journal of advanced research*, 20 Nov. 2024, doi:10.1016/j.jare.2024.11.027）。在研究过程中，王教授课题组利用国重平台的(1) **Olympus FV3000**激光共聚焦显微镜，通过高灵敏度和高速度成像，精细描绘了p65亚基核易位过程；(2) **Sony ID7000**全光谱流式细胞分析仪，在自动上样和简化补偿调节功能的优化方面大大提高了实验效率，实现了高效监测成纤维细胞周期阻滞和细胞凋亡情况。

Sony MA900
多功能全自动流式细胞分选仪
仪器位置：E101
负责老师：刘龙江
联系电话：31246566

Olympus FV3000
激光扫描共聚焦显微镜
仪器位置：E101
负责老师：杨建鸽
联系电话：31246565

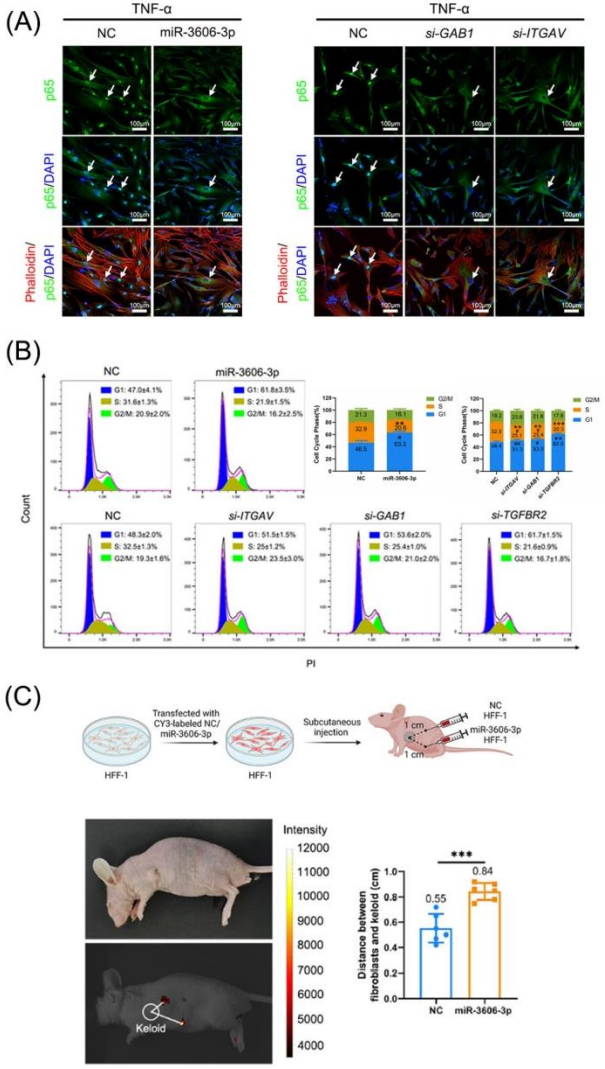

图1 (A)Olympus FV3000激光共聚焦显微镜拍摄的p65核易位的细胞免疫荧光图像。(B) 使用Sony ID7000全光谱流式细胞仪分析细胞周期变化。(C)小动物成像仪分析瘢痕疙瘩小鼠的成纤维细胞迁移情况。

Fibroblast abnormalities are crucial causes of skin fibrosis, including systemic sclerosis (SSc) and keloids. However, their mechanisms, including underlying microRNA regulatory mechanisms, remain elusive. Multiple abnormal activation of fibroblasts was found to be involved in inflammation, migration, proliferation and collagen deposition. miR-3606-3p can target ITGAV/GAB1/TGFBR2-mediated integrin/FAK,AKT/ERK, and SMAD pathways to regulate these abnormalities to alleviate skin fibrosis. The multiple targets and pleiotropic effects of miR-3606-3p are expected to be the intervention target for skin fibrosis.

In this study, the Olympus FV3000 laser confocal microscope provided multi-color full spectrum, high sensitivity and high resolution imaging conditions, which allowed clear outline of the cytoplasm and nucleus morphology and observation of key subunit activation and nuclear translocation. The Sony ID7000 full-spectrum flow cytometry analyzer could achieve high-throughput sample processing of up to 24 5mL test tubes, which simplified the operation steps of the experiment and improved the efficiency of the experiment. The results showed that the inhibitory effect of miR-3606-3p on fibroblast proliferation was achieved by cell cycle arrest at G1 phase. The real-time observation of small animal imager showed that the migration speed of fluorescein-labeled fibroblasts overexpressing miR-3606-3p was significantly inhibited in keloid mice.

成纤维细胞异常是系统性硬化症 （SSc） 和瘢痕疙瘩等皮肤纤维化疾病的关键原因，但具体的成纤维异常特征及其表观调控机制尚不完全清晰。本研究发现成纤维细胞多种异常活化参与炎症、迁移、增殖和胶原沉积；miR-3606-3p能够同时靶向ITGAV、GAB1和TGFBR2这三个基因，通过调节它们参与的integrin/FAK、AKT/ERK和SMAD信号通路，从而缓解皮肤纤维化症状。miR-3606-3p的多靶点、多效性有望成为皮肤纤维化的干预靶点。

在本研究中，Olympus FV3000激光共聚焦显微镜提供多色全光谱、高灵敏度和高分辨率成像条件，便于清晰勾勒胞质胞核形态并观察关键亚基活化及核易位；Sony ID7000全光谱流式细胞分析仪自动上样能实现多达24个5mL试管的高通量样本处理，简化了实验操作步骤并提高实验效率，继而分析发现miR-3606-3p对成纤维细胞增殖抑制作用是通过细胞周期阻滞在G1期实现的；利用小动物活体成像仪实时观测发现，荧光标记过表达miR-3606-3p的成纤维细胞的迁移速度在瘢痕疙瘩小鼠体内被显著抑制。

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细菌甲基转移酶Bioc的结构与功能研究

2024年12月20日复旦大学生科院甘建华研究员课题组联合浙江大学冯友军教授课题组于*Science Advances*发表题为“**A bacterial methyltransferase that initiates biotin synthesis, an attractive anti-ESKAPE druggable pathway**”的研究长文。在研究过程中，课题组利用了国重平台的蛋白质高效液相纯化系统以及晶体筛选工作站，解析了鲍曼杆菌甲基转移酶BioC与SAM复合物的晶体结构。蛋白质纯化系统提供了快速高效的纯化条件，保证了蛋白样品的质量，而结晶工作站则为晶体生长条件的大范围筛选提供了便利，大大提高了蛋白结晶成功的机率，为蛋白质结构的解析奠定了基础。

Gryphon结晶工作站
仪器位置：D214

蛋白质高效液相纯化系统
AKTA Micro
仪器位置：A201

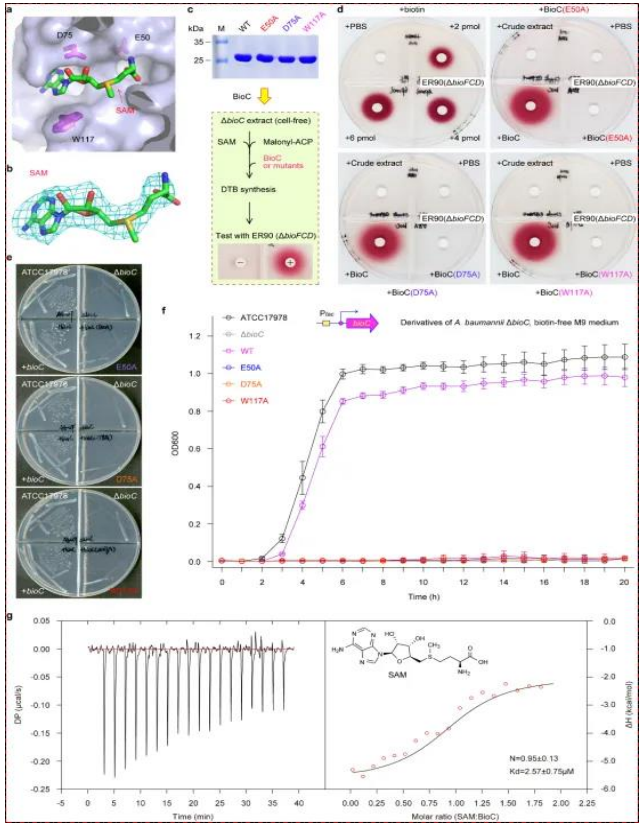

图1：细菌甲基转移酶BioC参与生物素合成的生化分析。a, b）BioC与底物SAM所形成复合物的晶体结构。c-g）基于BioC与SAM的结构信息，构建了一系列BioC突变体，并且通过细菌生长和体外生物素重建实验，对甲基化酶BioC与SAM分子的精细互作进行了验证。

The covalently attached cofactor biotin plays pivotal roles in central metabolism. The top-priority ESKAPE-type pathogens, Acinetobacter baumannii and Klebsiella pneumoniae, constitute a public health challenge of global concern. Despite the fact that the late step of biotin synthesis is a validated anti-ESKAPE drug target, the primary stage remains fragmentarily understood. We report the functional definition of two BioC isoenzymes (AbBioC for A. baumannii and KpBioC for K. pneumoniae) that act as malonyl-ACP methyltransferase and initiate biotin synthesis.The physiological requirement of biotin is diverse within ESKAPE pathogens. CRISPR-Cas9–based inactivation of bioC rendered A. baumannii and K. pneumoniae biotin auxotrophic. The availability of soluble AbBioC enabled the in vitro reconstitution of DTB/biotin synthesis. We solved two crystal structures of AbBioC bound to SAM cofactor (2.54 angstroms) and sinefungin (SIN) inhibitor (1.72 angstroms). Structural and functional study provided molecular basis for SIN inhibition of BioC. We demonstrated that BioC methyltransferase plays dual roles in K. pneumoniae infection and A. baumannii colistin resistance.

本研究通过广泛筛选获得了可溶性表达的鲍曼杆菌BioC蛋白质样品，解析了BioC与SAM复合物的晶体结构，阐明了BioC结合SAM的分子机制。此外，本研究还通过结构解析的手段阐明了SAM类似物竞争性结合BioC的分子机制。结构指导的蛋白质定点突变、细胞生长以及生物素重构实验均证明BioC参与生物素的合成途径，对ESKAPE病原菌粘菌素耐药的形成至关重要。除具体的催化机制外，本研究还为抵御超级耐药菌感染的新型抗菌药物研发提供了新的靶点。

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