NEW METHODS FOR ANTIBIOTICS DISCOVERY AND MOLECULAR MECHANISM CHARACTERIZATION

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THE URGENCY OF NEW ANTIBIOTICS REQUIRES NEW METHODOLOGIES

- High-throughput in vitro screens are consolidated approaches to discover antibiotic compounds, but the current profiling methods are limited in either the number of measurable parameters or throughput.

- Also de novo predictions of compound functionality with unconventional Modes of Action (MoAs) is fundamental to challenge the problem of new antibiotic-resistant bacteria and for the finding of effective multidrug combinations against bacterial infections.

- Molecular profiling of small molecules offers invaluable insights into the function of compounds and allows for hypothesis generation about small-molecule direct targets and secondary effects **partially bypassing the request for animal research**.

ARTICLES
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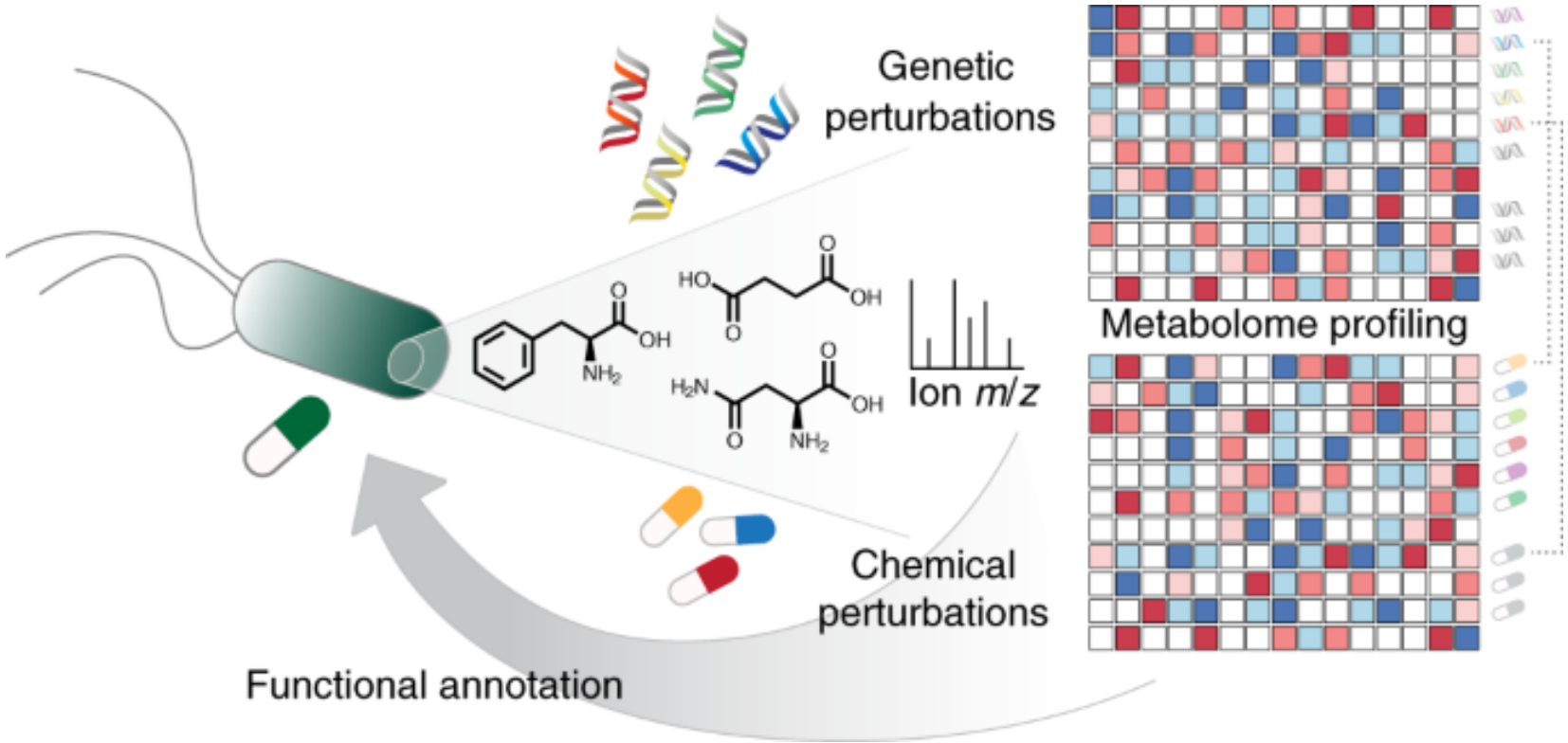
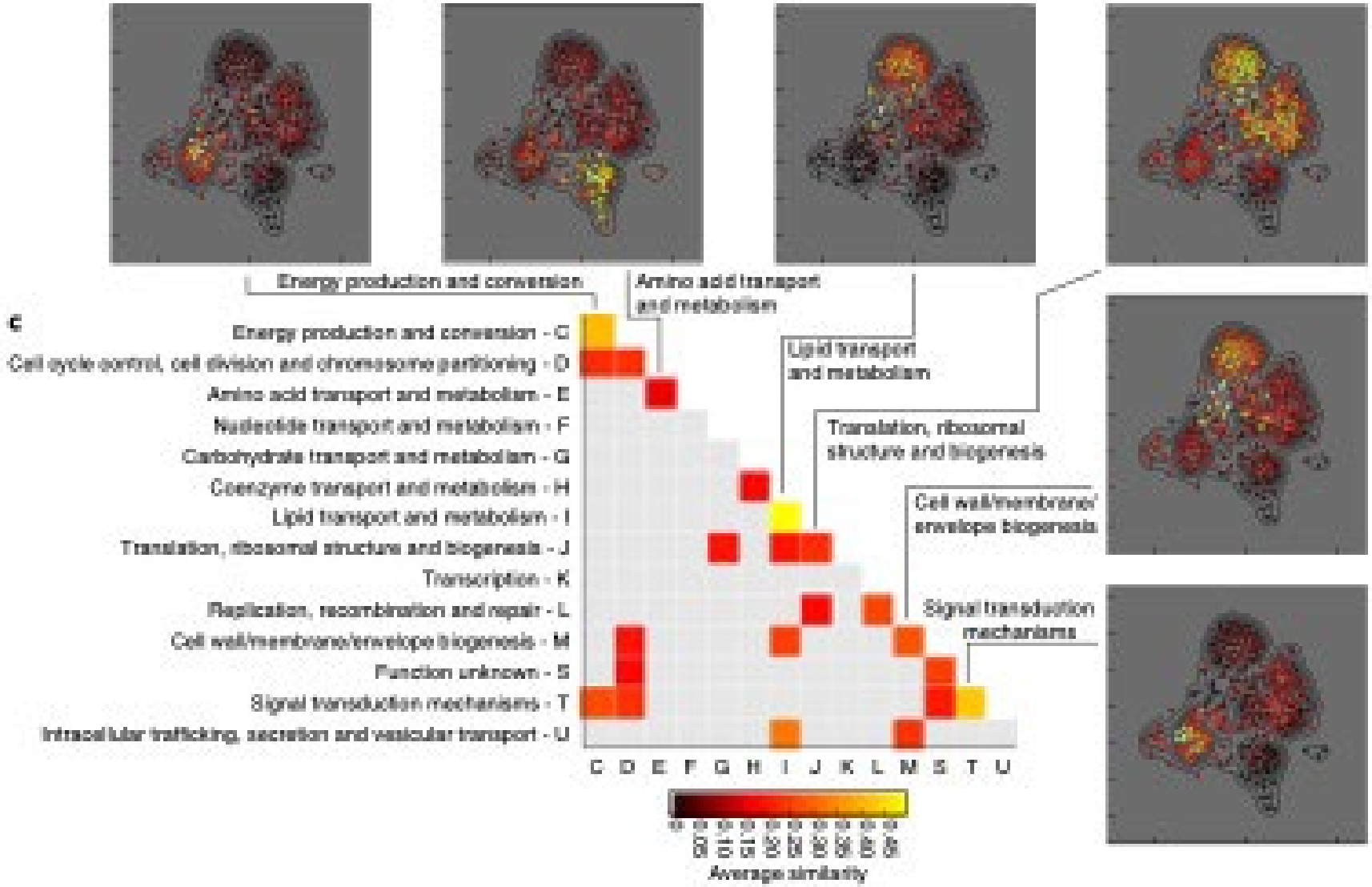
Combining CRISPRi and metabolomics for functional annotation of compound libraries

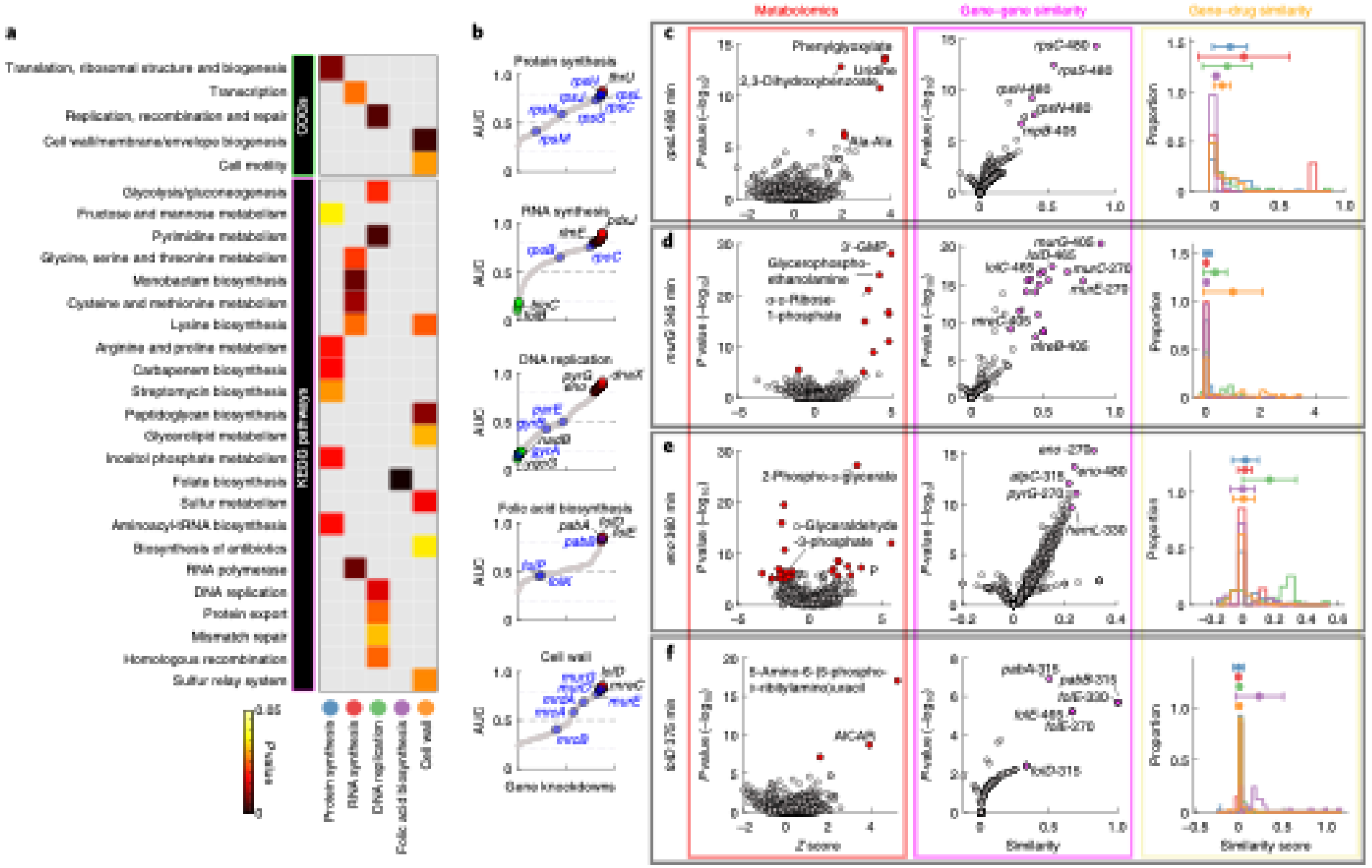
Miquel Anglada-Girotto[1,4], Gabriel Handschin[1,4], Karin Ortmayr[1], Adrian I. Campos[1], Ludovic Gillet[1], Pablo Manfredi[2], Claire V. Mulholland[3], Michael Berney[3], Urs Jenal[2], Paola Picotti[1] and Mattia Zampieri[1]✉



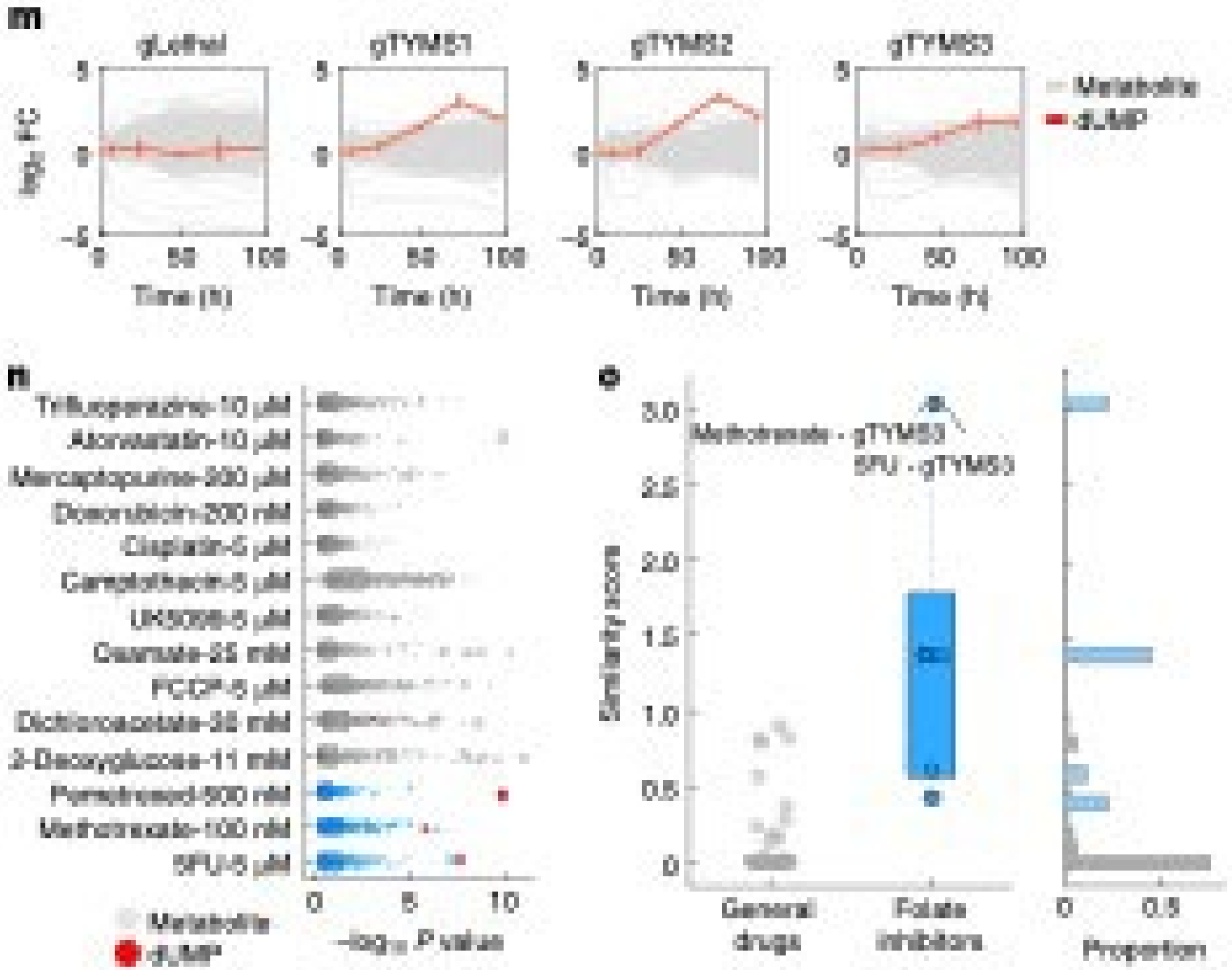
GENETIC PERTURBATIONS INDUCE MEASURABLE METABOLIC CHANGES



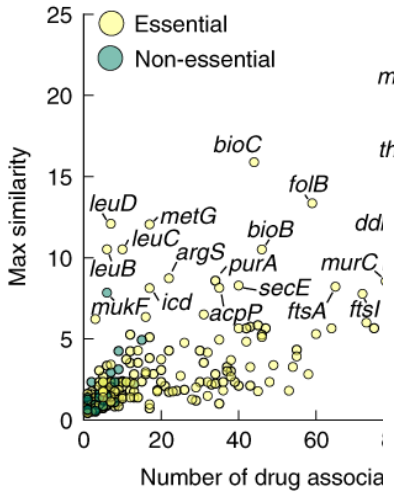
FUNCTIONAL ASSOCIATIONS BETWEEN GENE KNOCKDOWNS AND MAJOR ANTIBIOTIC CLASSES

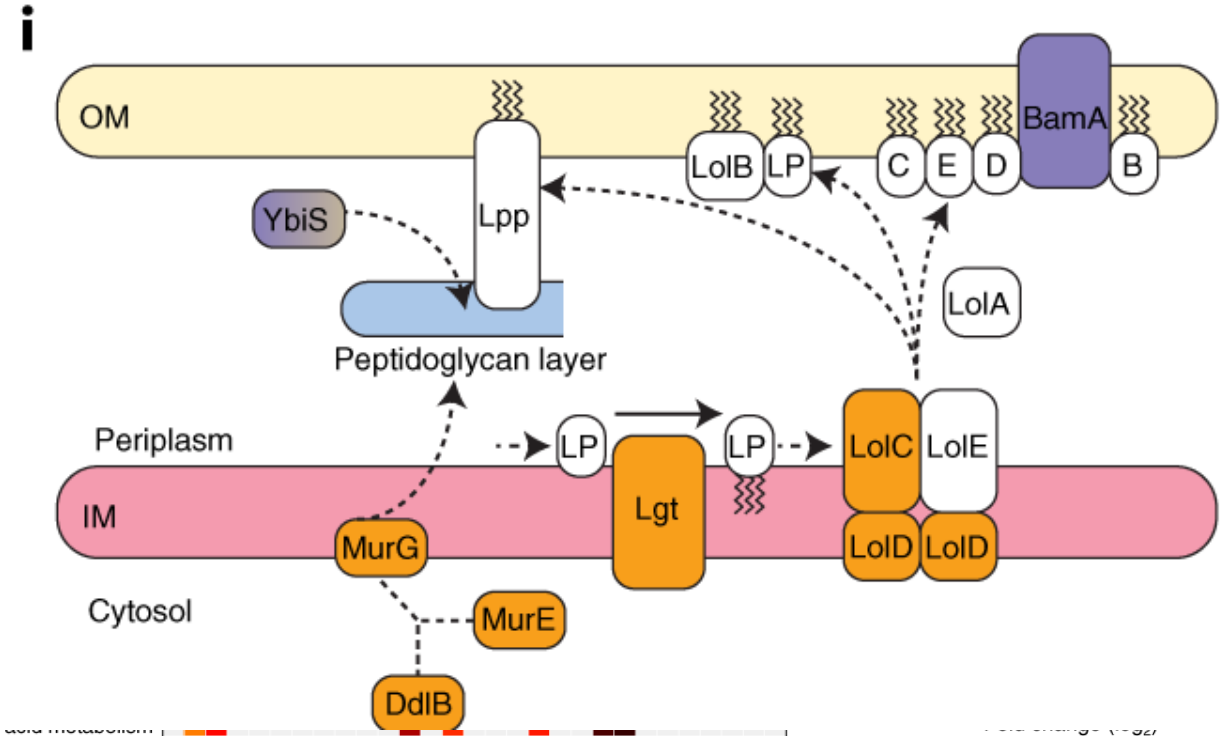


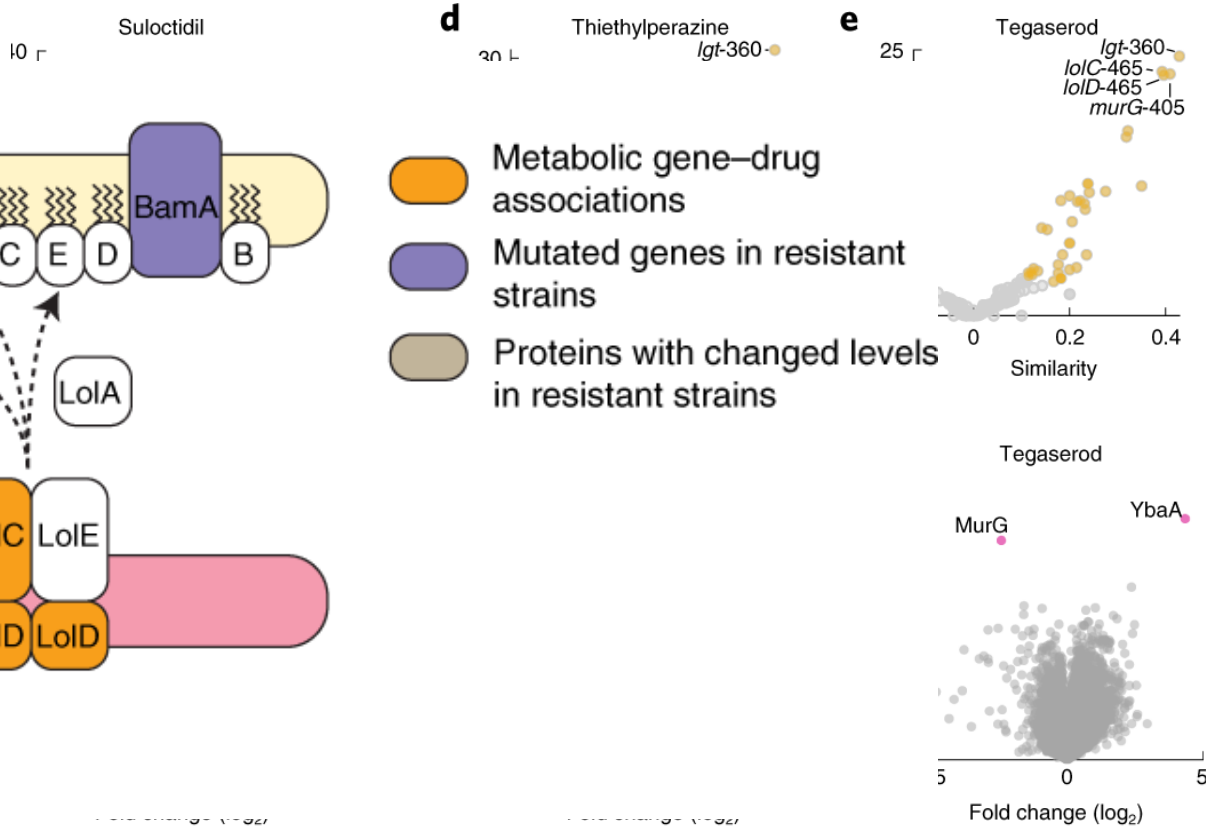
METABOLIC PROFILING OF CHEMICAL AND GENETIC PERTURBATIONS IN HUMAN CELLS



DE NOVO PREDICTION OF ANTIMICROBIAL MoAs







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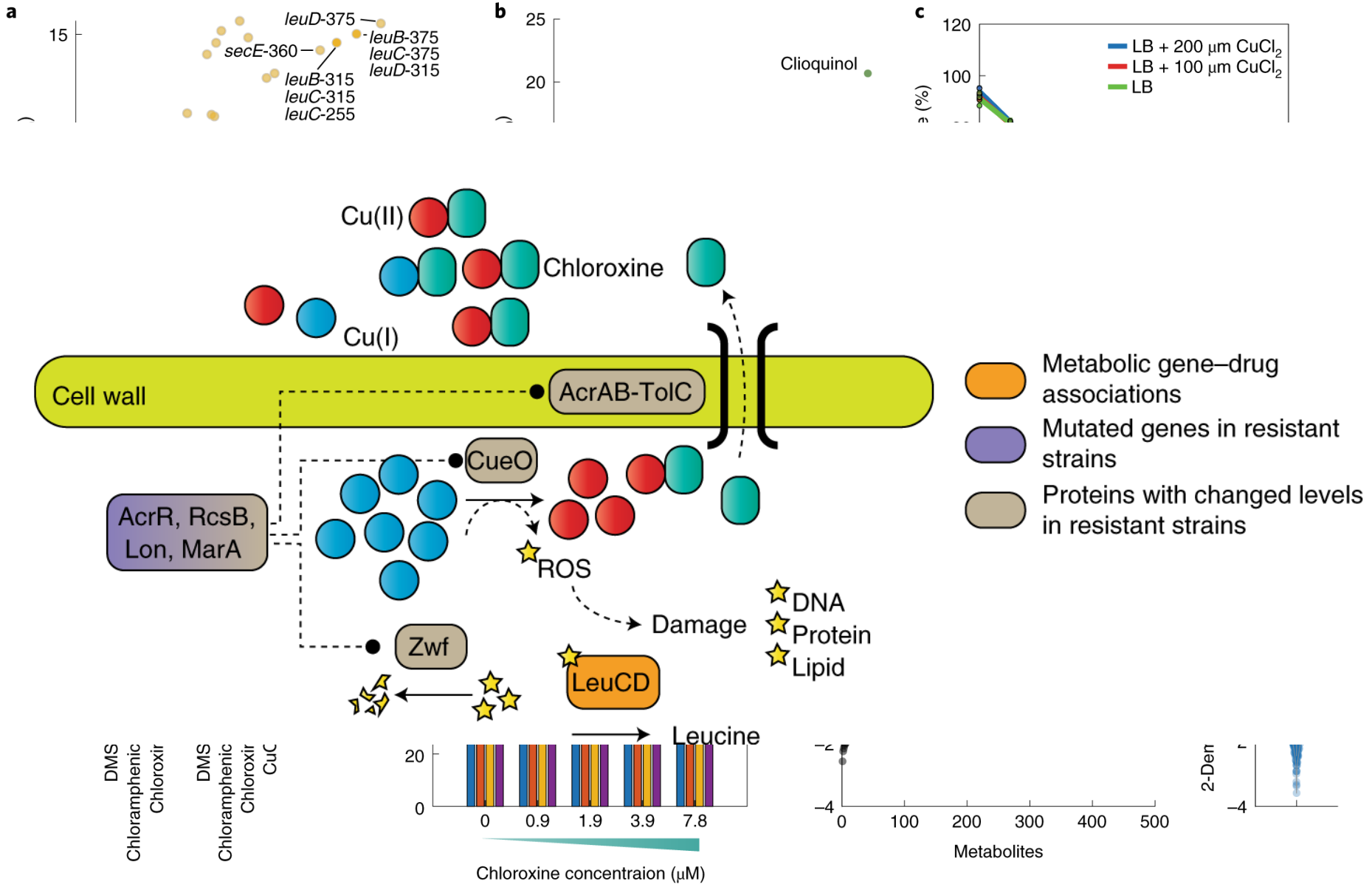

Conclusions

- The combined gene–drug metabolic profiling approach provides a high-throughput platform for unbiased and rapid functional annotation of compound libraries
- Directly measuring the metabolic consequences of drug treatment can unravel mechanistic insights on the MoA of compounds that do not directly bind to proteins
- This approach is highly flexible and can be applied in virtually any type of system or conditions

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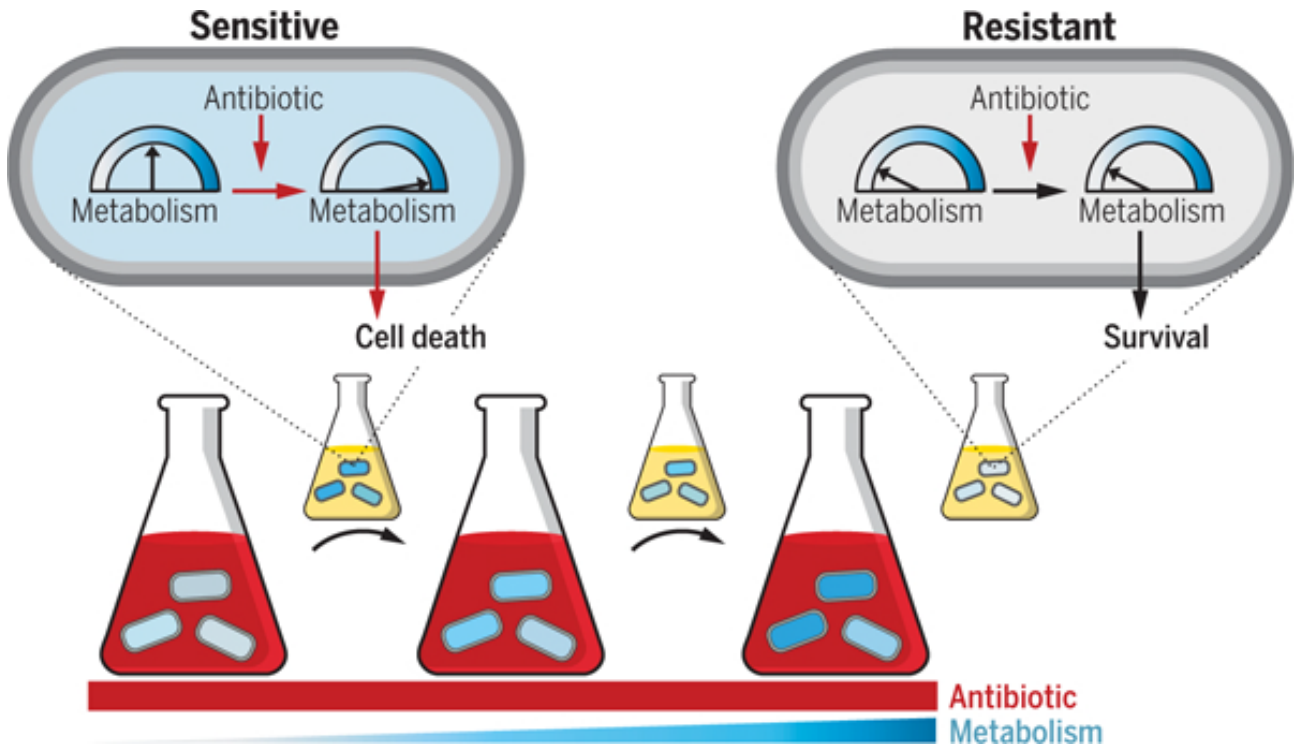
Clinically relevant mutations in core metabolic genes confer antibiotic resistance

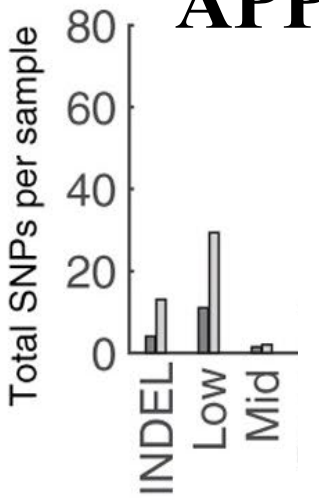
Allison J. Lopatkin[1,2,3,4,5,6], **Sarah C. Bening**[1,2], **Abigail L. Manson**[2], **Jonathan M. Stokes**[1,2,3], **Michael A. Kohanski**[7], **Ahmed H. Badran**[2], **Ashlee M. Earl**[2], **Nicole J. Cheney**[8,9], **Jason H. Yang**[8,9], **James J. Collins**[1,2,3,10,11,*]

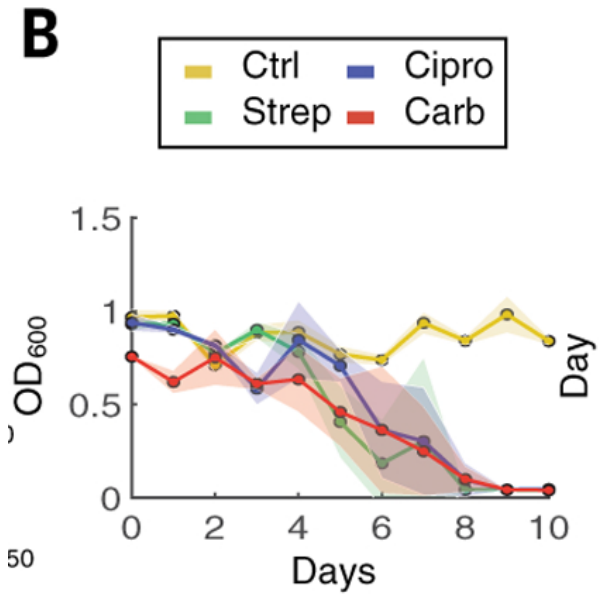


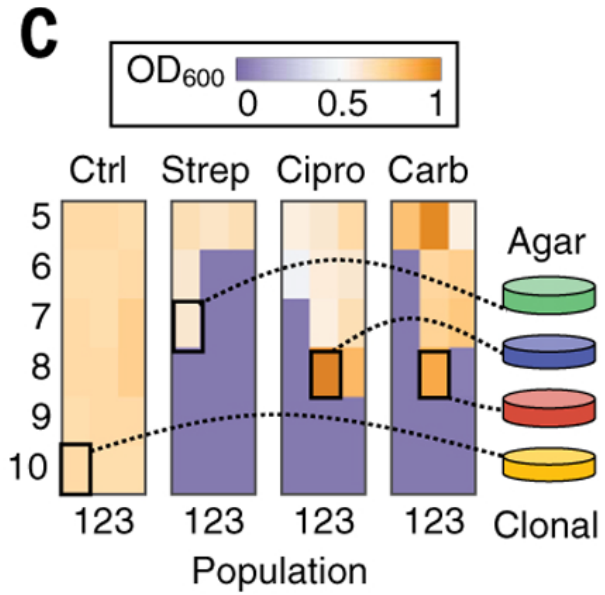
EVOLVING ANTIBIOTIC RESISTANCE USING THE CLASSICAL APPROACH

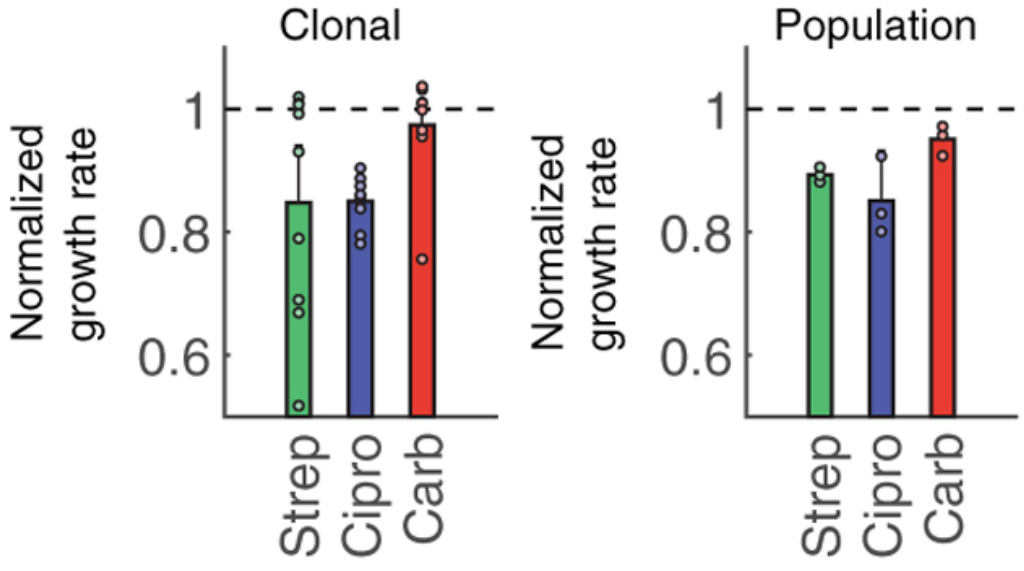
Drug	Time point	
	Mid	End
Strep	P:3, C:0	P:3, C:12
Cipro	P:3, C:0	P:3, C:12
Carb	P:3, C:0	P:3, C:12
Ctrl	P:3, C:0	P:3, C:4

P: populations C: clones









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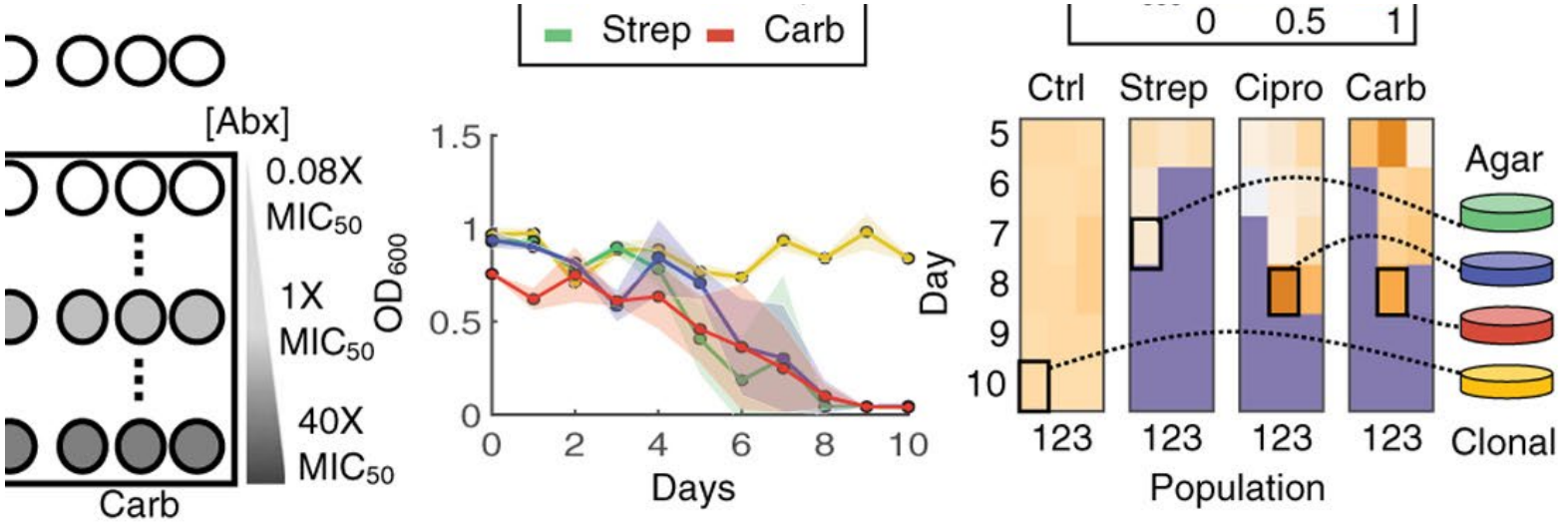
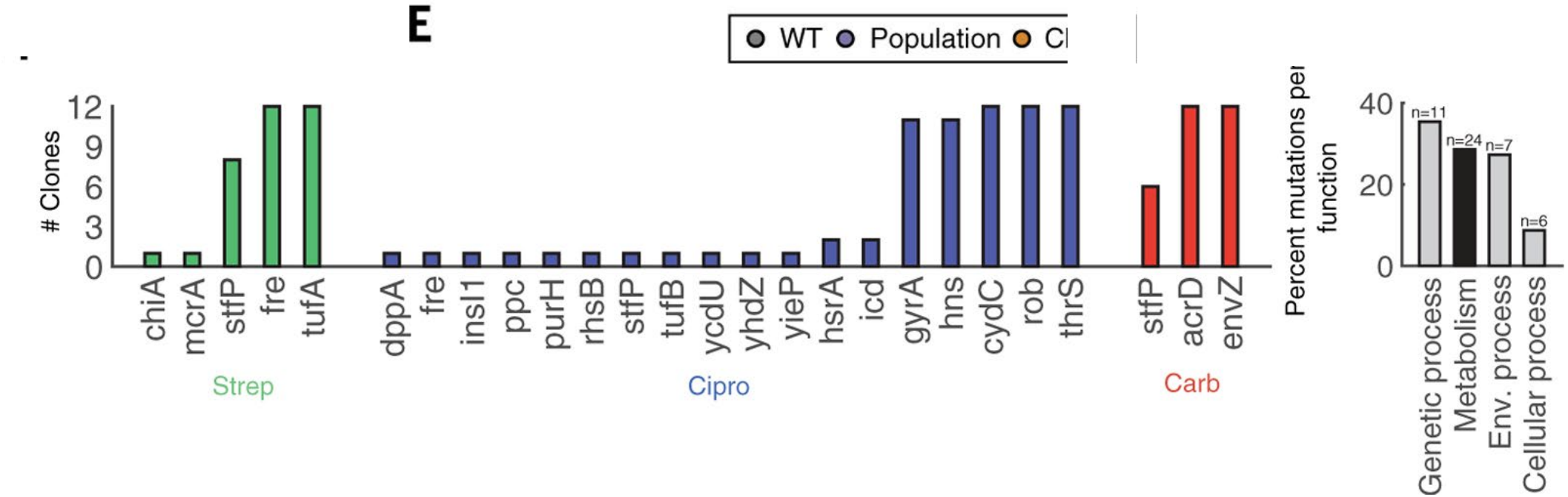
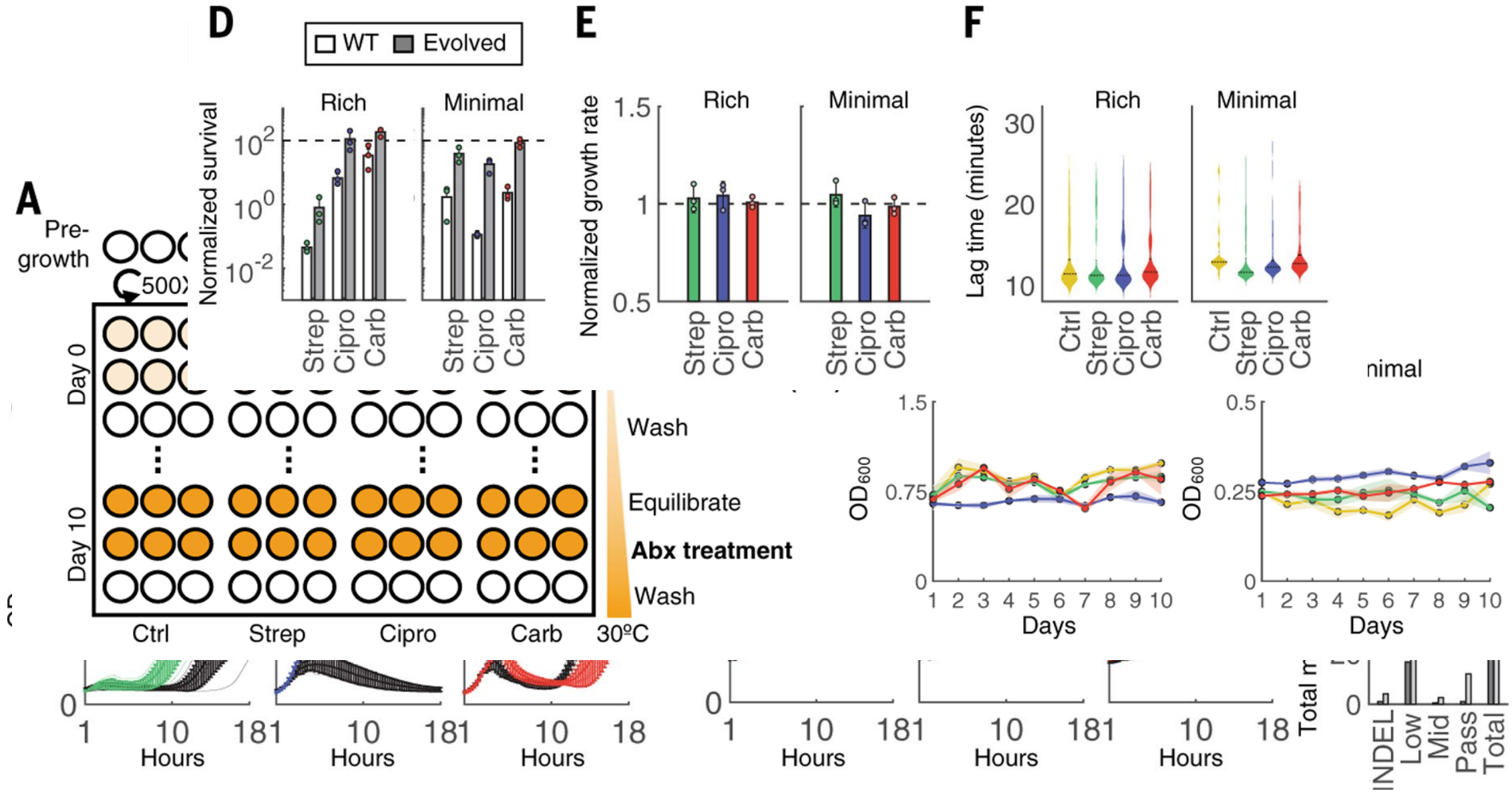
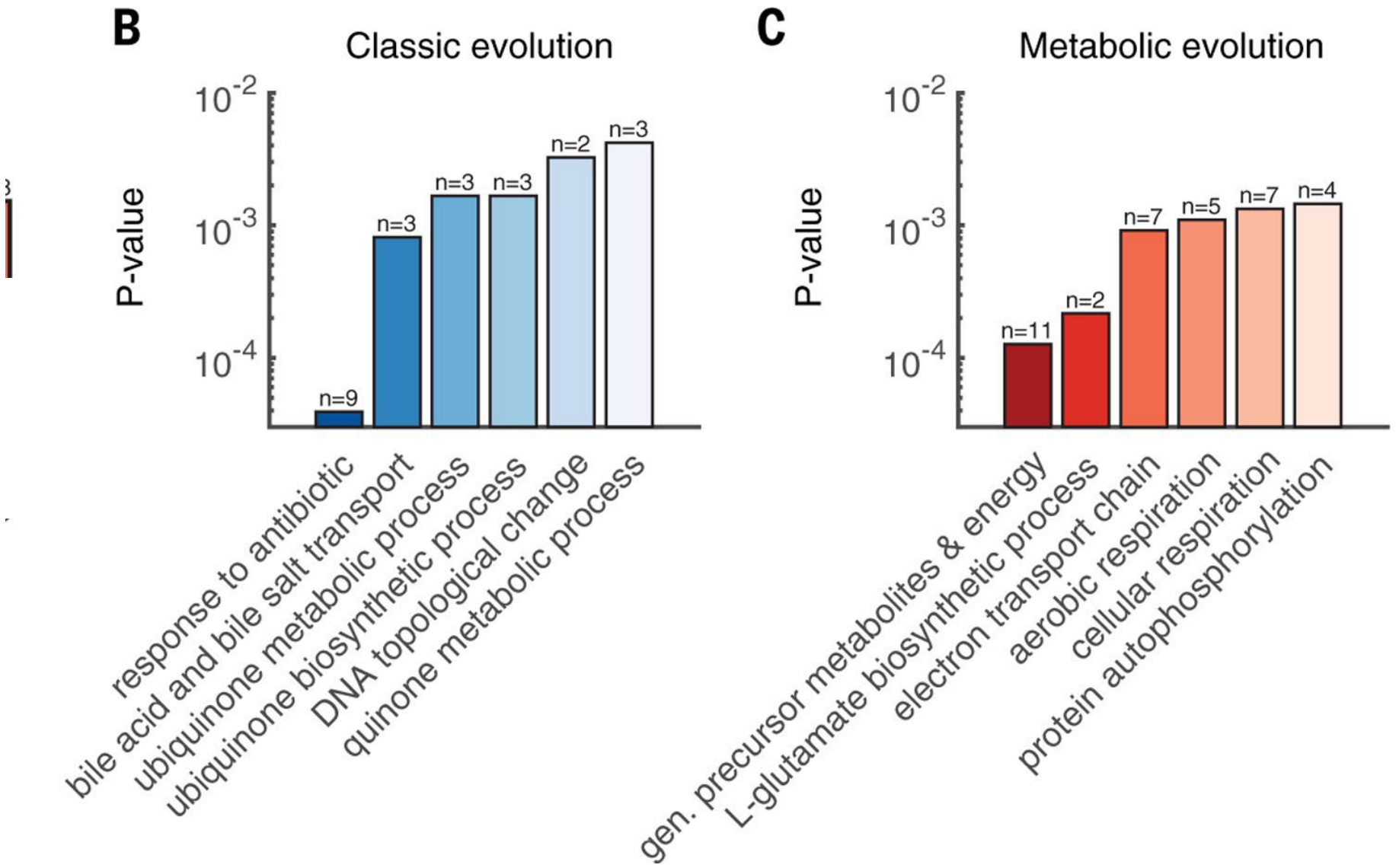




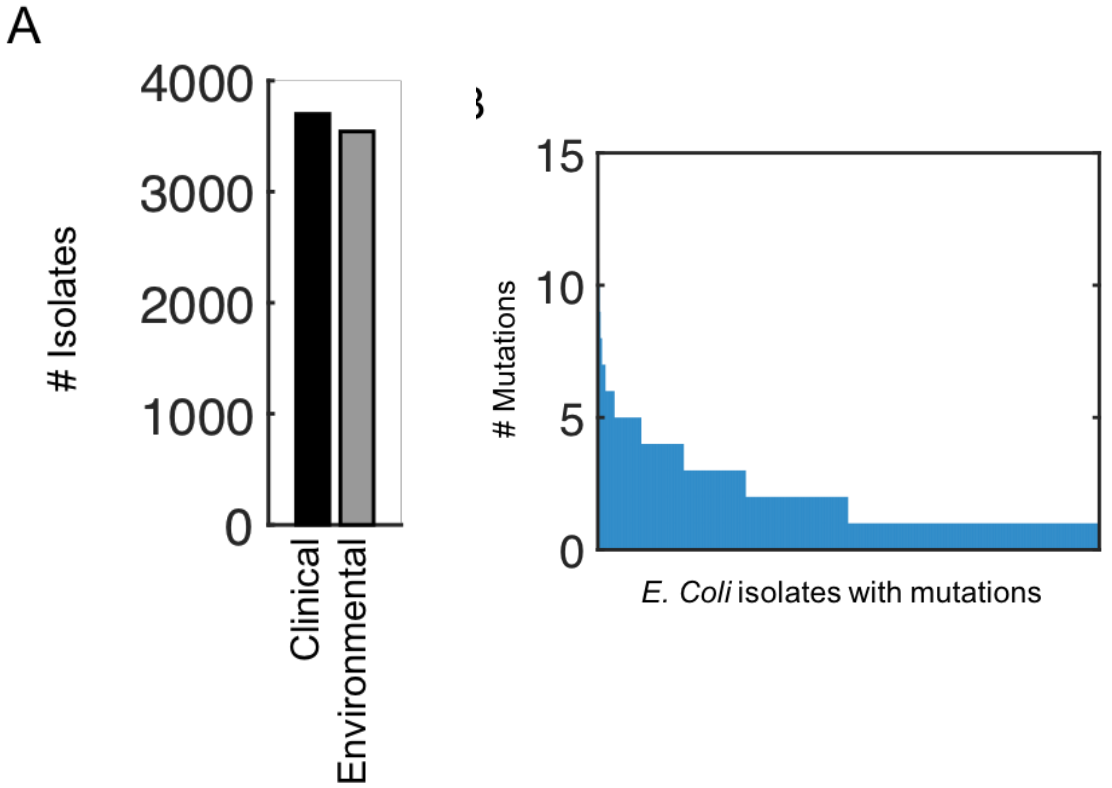
EVOLVING ANTIBIOTIC RESISTANCE USING THE METABOLIC APPROACH

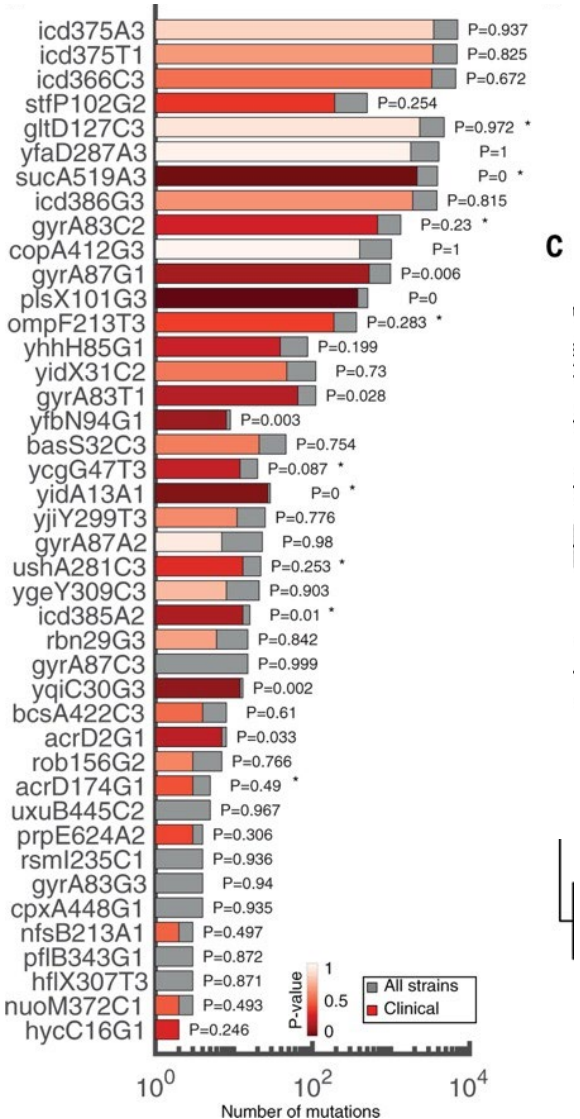


GENE ONTOLOGY ENRICHMENT ANALYSIS DIFFERS BETWEEN EVOLUTIONS

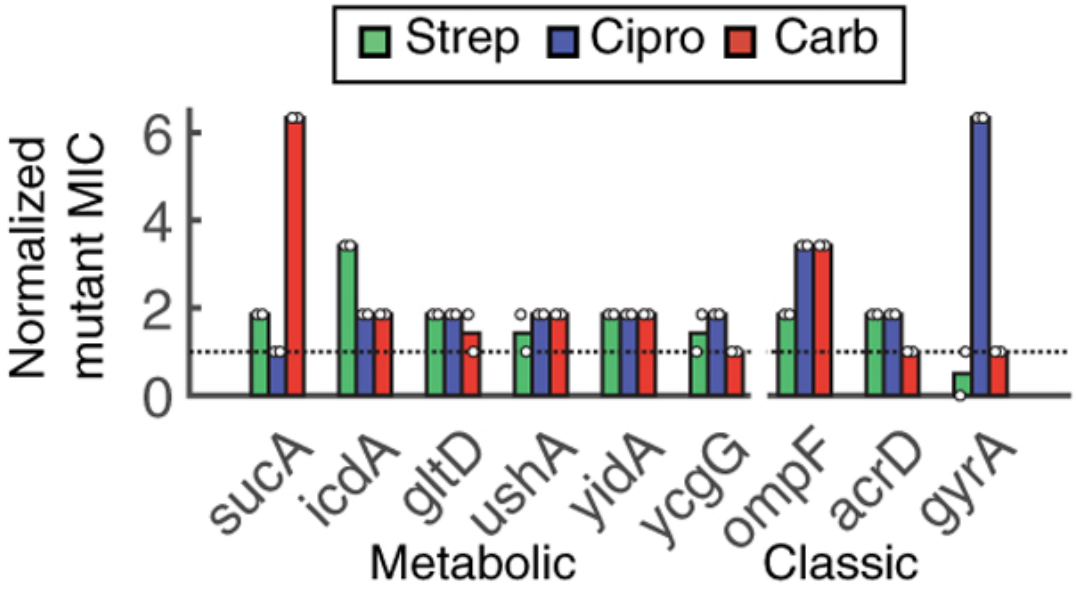


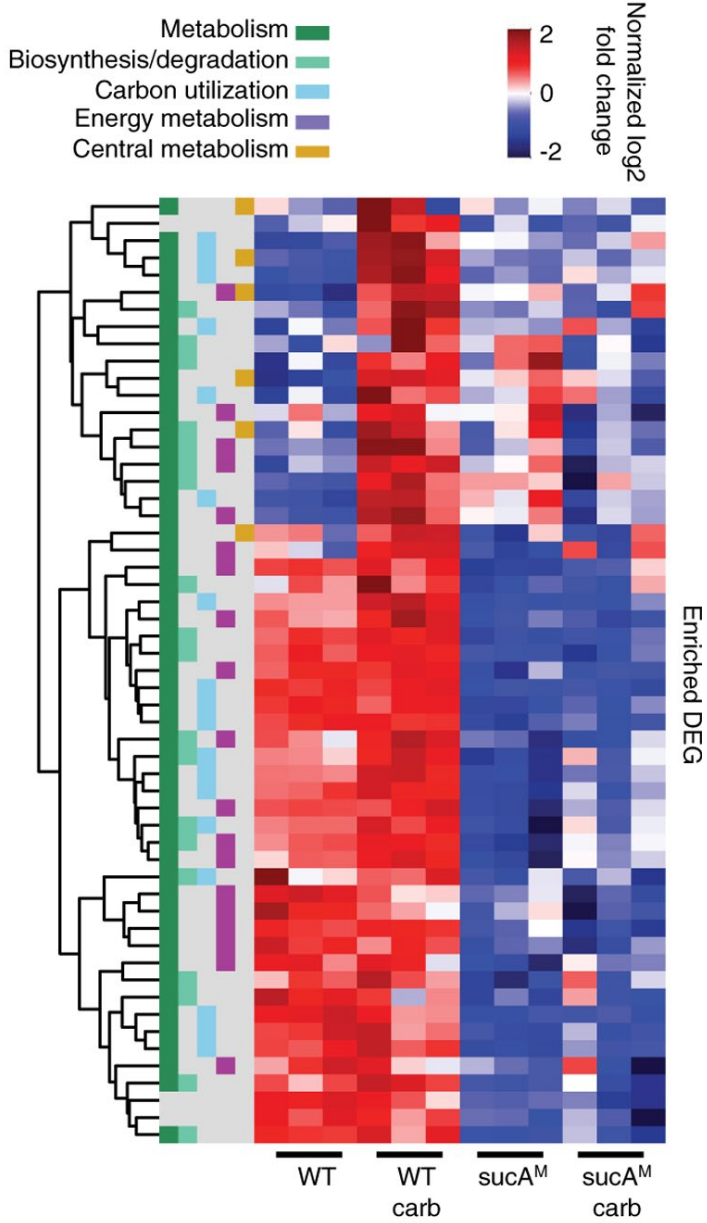
METABOLIC MUTATIONS ARE HIGHLY PREVALENT IN E. COLI GENOMES AND CONFER RESISTANCE

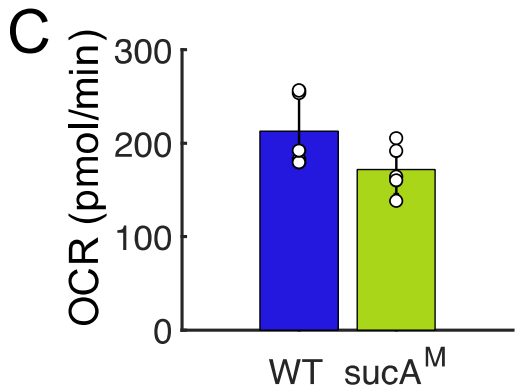


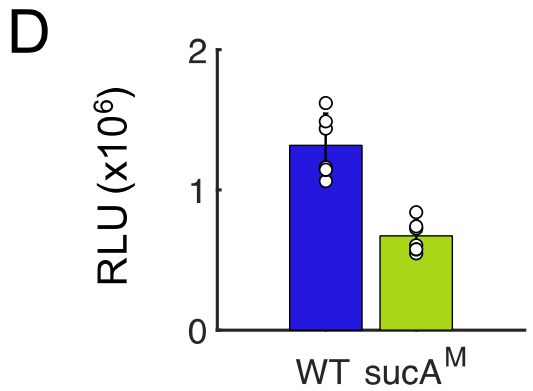


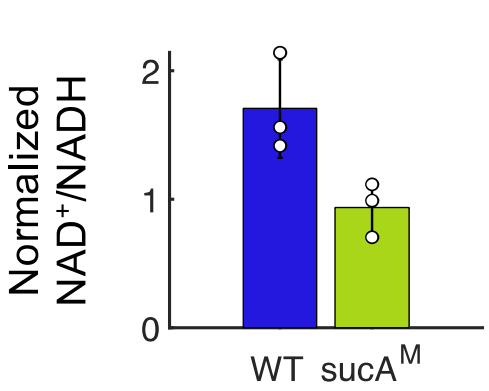
KNOCKOUT AND OVEREXPRESSION CONFIRM GENETIC UNDERPINNINGS OF ANTIBIOTIC RESISTANCE











Conclusions

- Metabolic mutations arise in response to antibiotic treatment, and these mutations confer resistance and are highly prevalent in clinical pathogens, suggests that canonical mutations may not be as representative, nor the mechanisms as comprehensive, as previously thought.

- Implementing new protocols to increase the accessible evolutionary pathways could facilitate the discovery of new resistance mechanisms and thereby enhance our ability to limit the development and spread of antibiotic resistance.

Thank you for your attention