

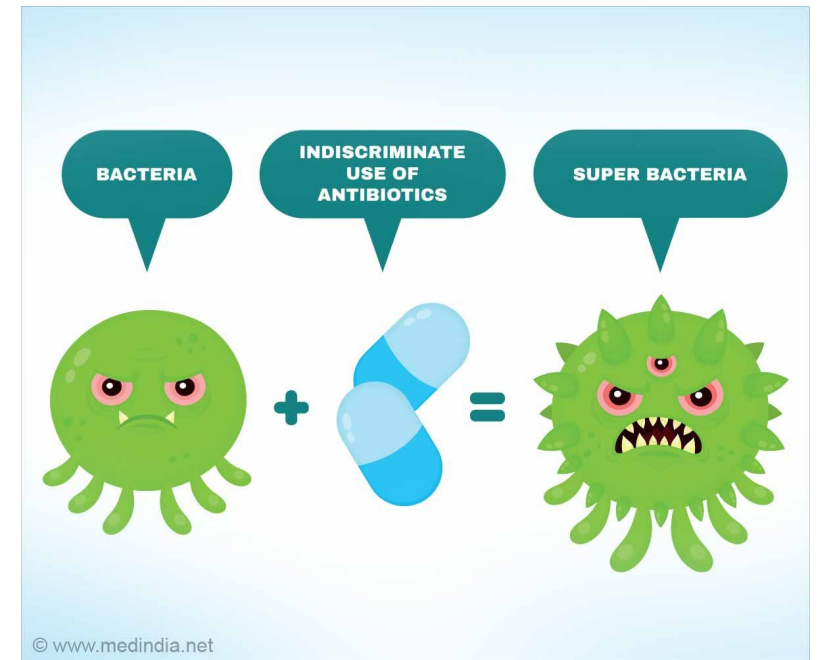
Lois Armendariz

Applications of *Caenorhabditis elegans* for  
Identification of Treatments Against  
Antimicrobial-Resistant Bacteria

Rice University  
Natasha Kirienko & Michael Lorenz  
Molecular Basis of Infectious Disease

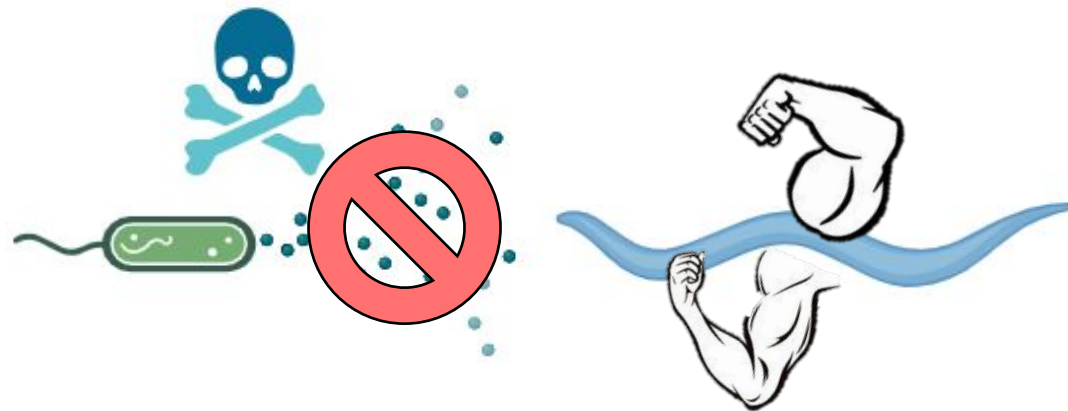
# The Rise of Multidrug Resistance in Hospital Associated Infections (HAIs)

- The misuse of antimicrobials has increased, leading to a faster rate of antimicrobial resistance (especially in hospitals).
- Common sources of HAIs:
  1. *Acinetobacter baumannii*
  2. *Burkholderia cepacia* complex
  3. *Pseudomonas aeruginosa*
  4. *Clostridium difficile*
  5. Vancomycin resistant Enterococci



# There is a need for new approaches to tackle antimicrobial resistance (AMR)

- Development and spread of AMR have seen a dramatic increase
- Over 2 million AMR infections occur yearly
- Relying on current or new antimicrobials will not solve this crisis
- One solution is the idea of modulating host-pathogen interaction



# *Caenorhabditis elegans* can be used as a model for host-pathogen interaction studies

- Small & transparent
- Short generation time
- Fully sequenced genome
- Shares conserved innate immune features with mammals
- Can be used in whole-animal pathogenesis models
- Pathogens use the same virulence mechanisms to infect humans and *C. elegans*



Adapted from Shivers et. al., (2010)

# Conventional antibiotics display reduced efficacy against MDR isolates

**A**

	AMK	AMP	AMS	AZM	Cfz	Cpe	Cfx	Caz	Cax	CIP	Etp	GEN	Lvx	Mer	F/M	TZP	TET	TOB	T/S
PA14	S	R	R	S	R	S	R	S	R	S	R	S	S	S	R	S	R	S	R
PA 2-61	S	R	R	I	R	S	R	R	R	S	R	R	S	S	R	S	R	I	R
PA 2-72	S	R	R	S	R	S	R	S	R	S	R	S	S	S	R	S	R	S	R
PA 2-88	S	R	R	S	R	S	R	S	R	I	R	R	I	S	R	S	R	S	R
PA 3-22	S	R	R	S	R	S	R	S	R	I	R	R	I	S	R	S	R	I	R

## Virulent Isolates

PA14

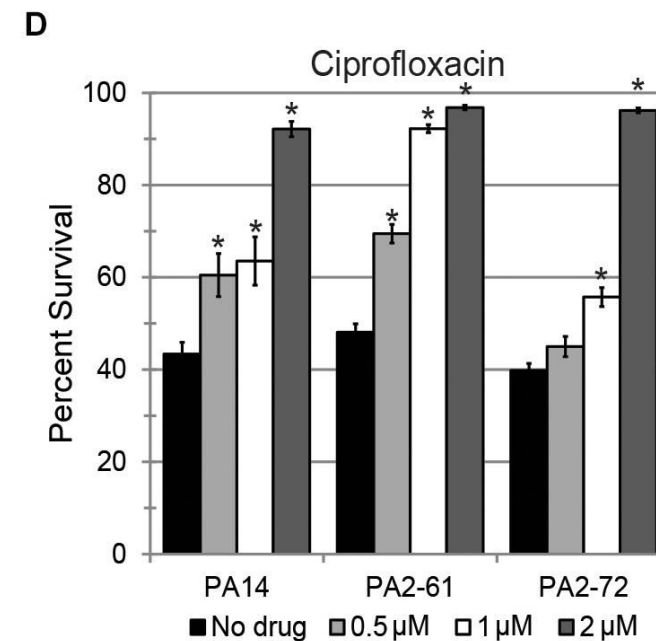
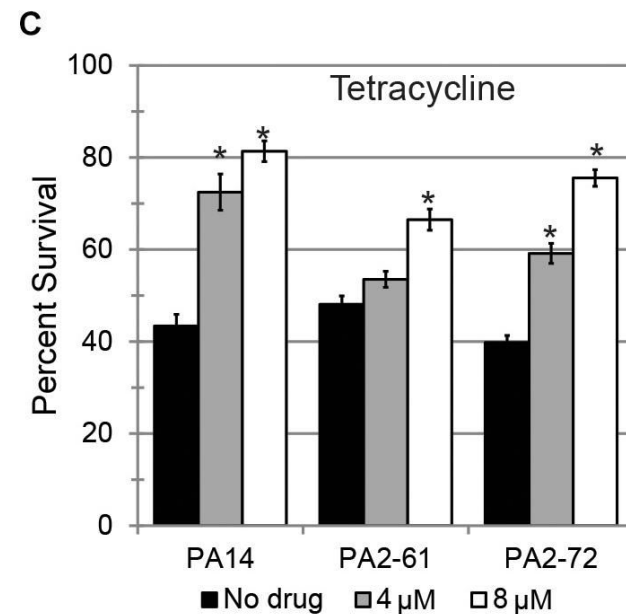
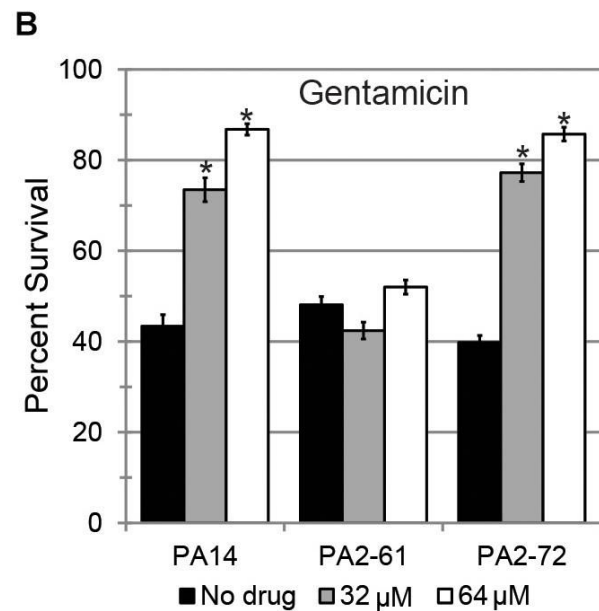
PA2-61

PA2-72

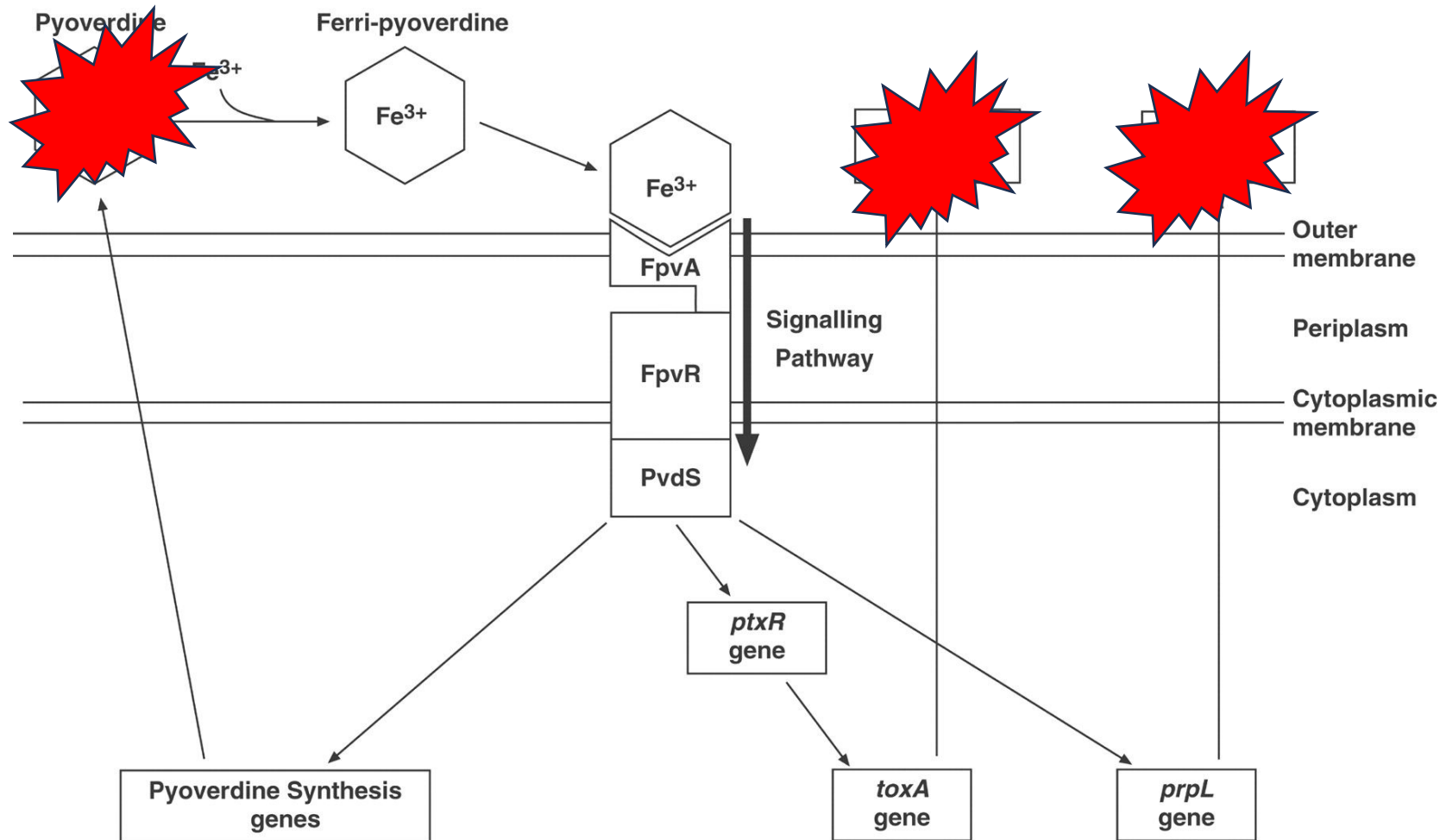
## Avirulent Isolates

PA2-88

PA3-22

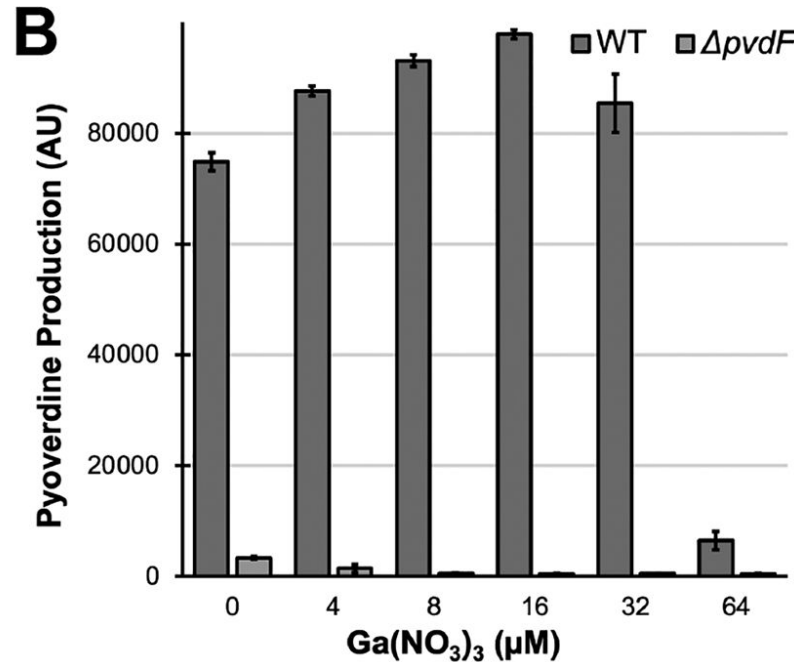
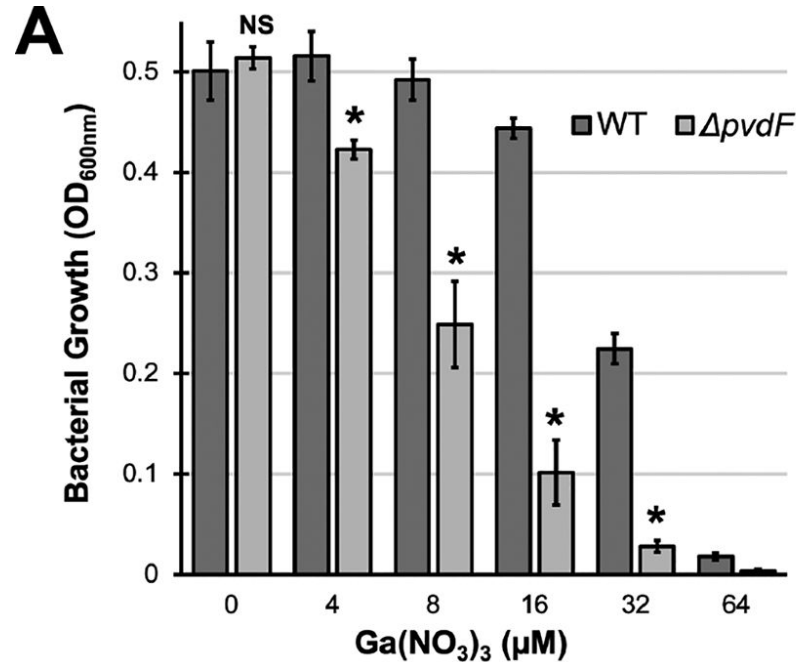


# Targeting bacterial virulence determinants as an alternative approach



- ❑ Pyoverdine is *P. aeruginosa*'s major siderophore
- ❑ Pyoverdine is indispensable for virulence in murine and worm model
- ❑ Targeting pyoverdine production would lead to inhibition of exotoxin A and proteases
- ❑ Pyoverdine is a good target

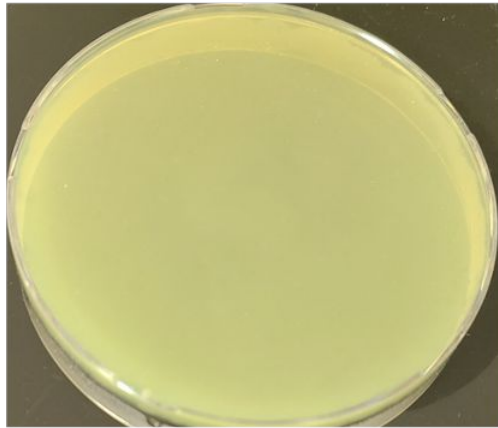
# Gallium Nitrate [Ga(NO<sub>3</sub>)<sub>3</sub>]: a popular antipseudomonal treatment has its limitations



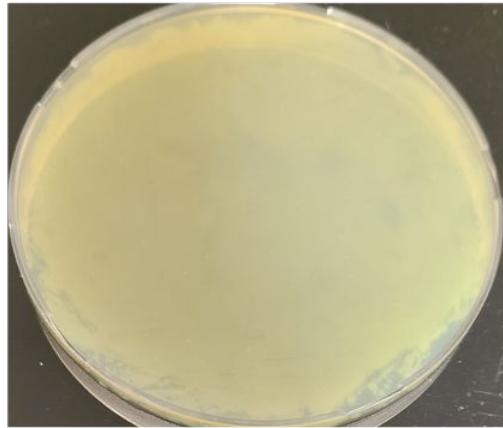
- Ga(NO<sub>3</sub>)<sub>3</sub> acts as a ferric iron mimetic and is subject to sequestration by siderophores
- Binding of Ga(NO<sub>3</sub>)<sub>3</sub> by pyochelin and pyoverdine elicit different results
- Pyoverdine production leads to decreased susceptibility of *P. aeruginosa* to Ga(NO<sub>3</sub>)<sub>3</sub>

# Synergistic Therapy – a Potential Solution?

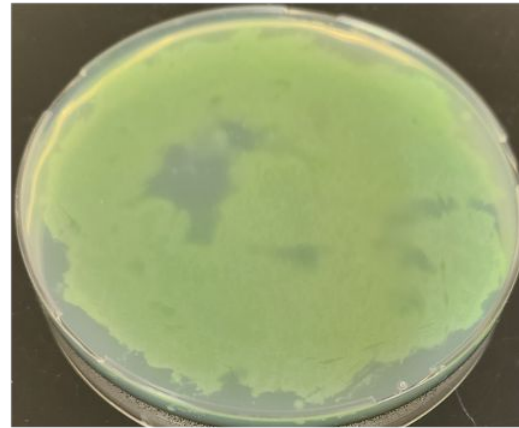
36 h



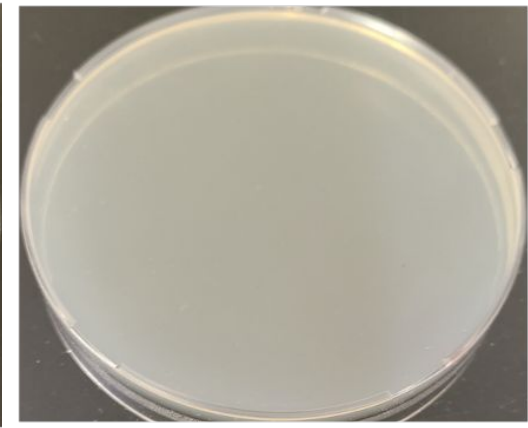
Media



5FC 250  $\mu$ M

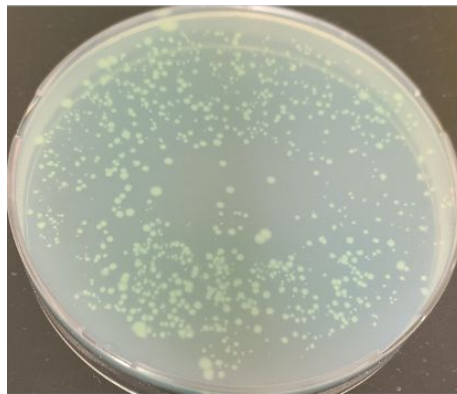


Ga 150  $\mu$ M

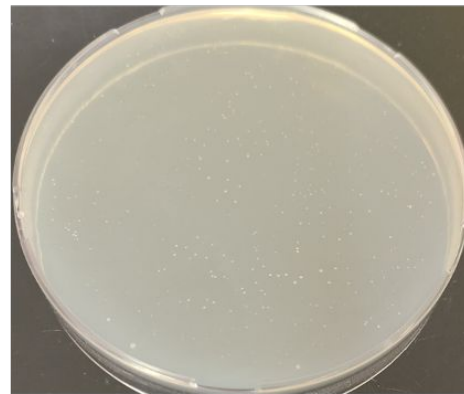


Ga 150  $\mu$ M; 5FC 250  $\mu$ M

60 h

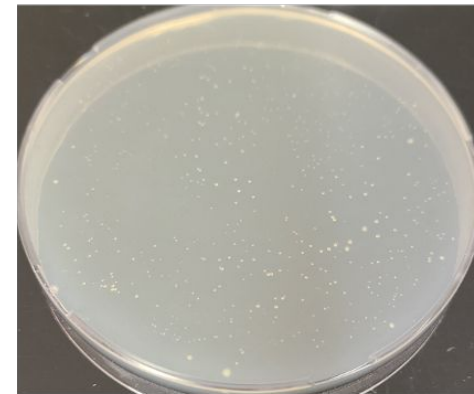


Ga 300  $\mu$ M



Ga 150  $\mu$ M; 5FC 250  $\mu$ M

84 h



Ga 150  $\mu$ M; 5FC 250  $\mu$ M

\*Ga: Gallium Nitrate

\*5FC: 5-Fluorocytosine

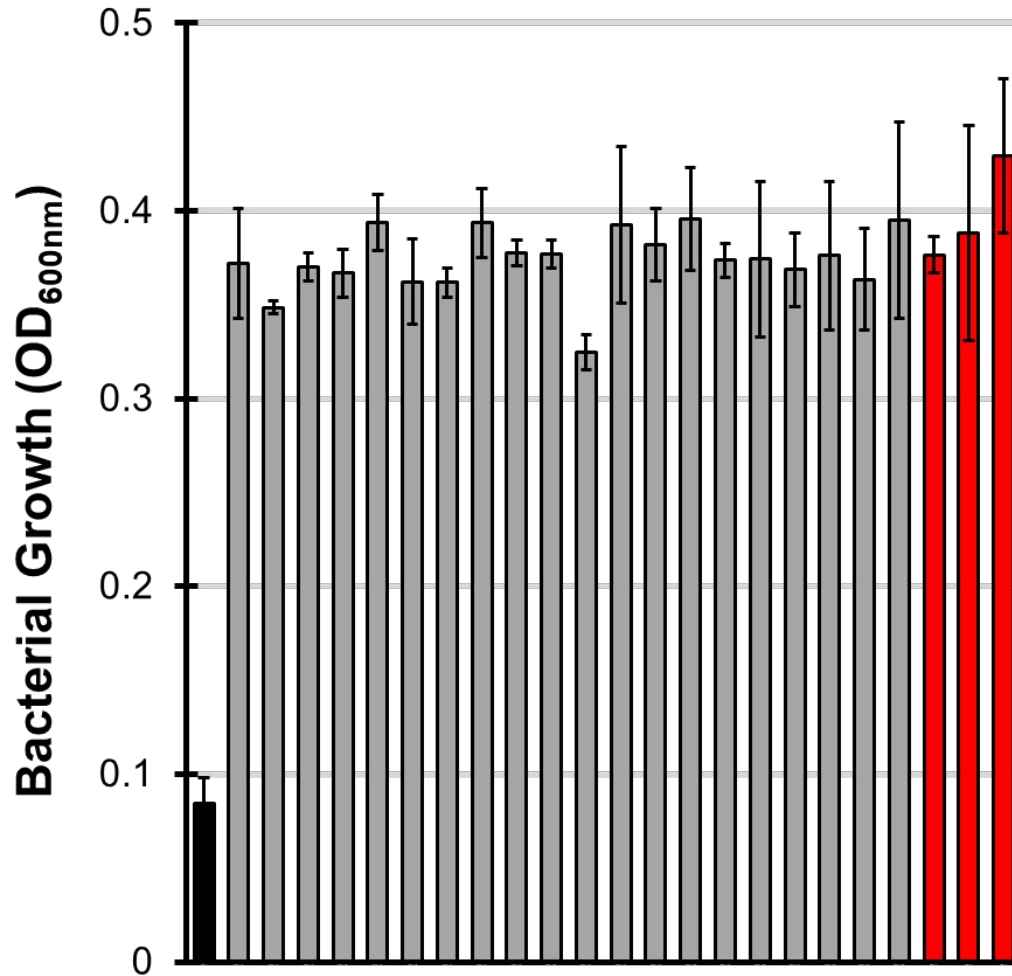


# Synergistic Therapy – a Potential Solution?

Growth in 64  $\mu\text{M}$  Gallium

Black: parental PAO1

Red: PAO1 adapted in 300  $\mu\text{M}$  Ga

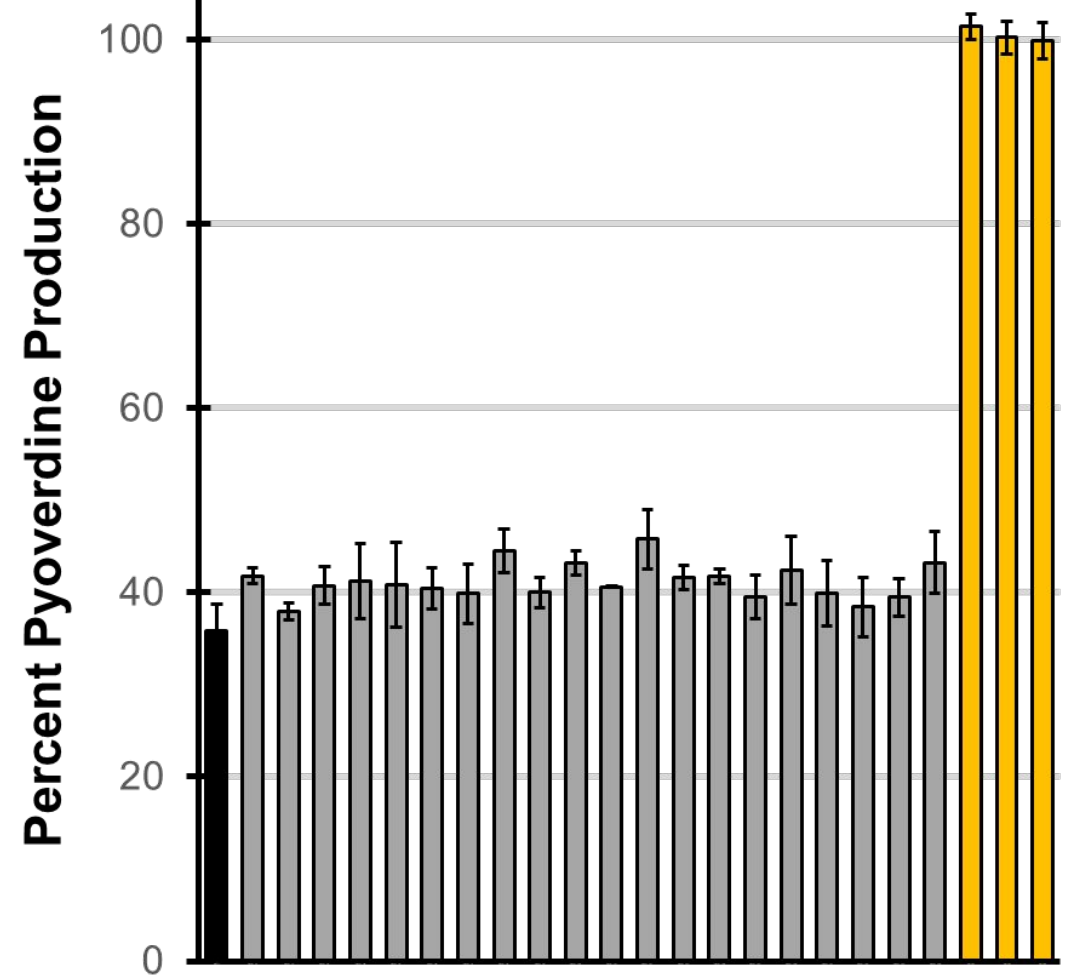


% pyoverdine production in the presence of

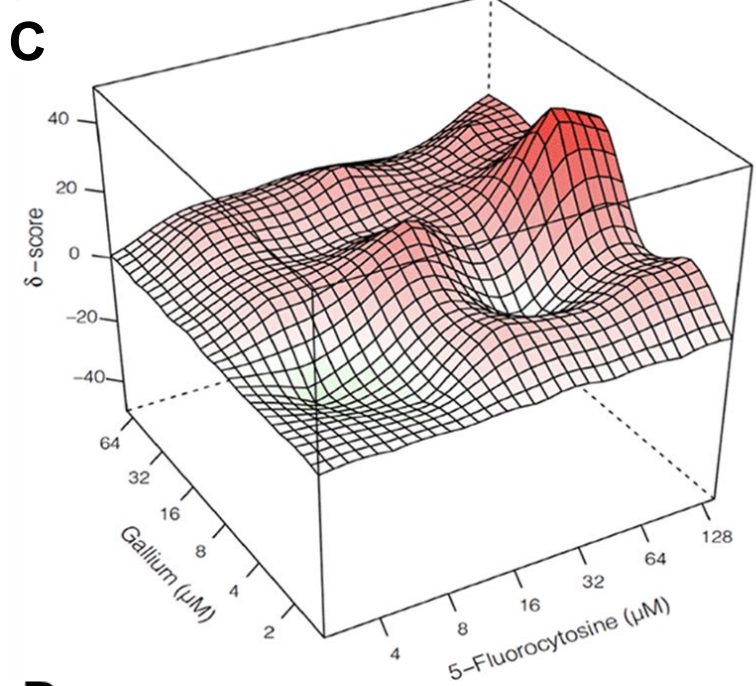
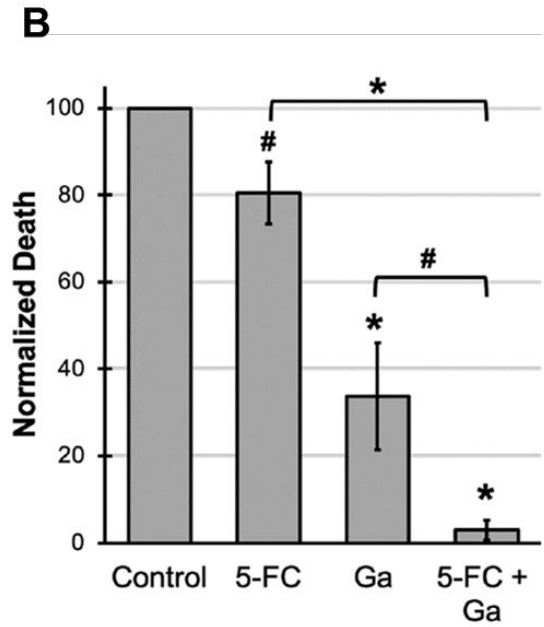
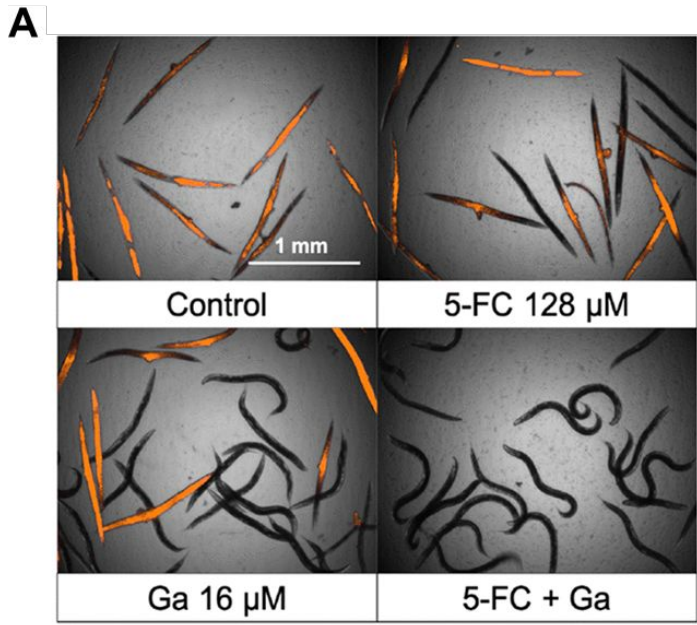
100  $\mu\text{M}$  5-FC

Black: parental PAO1

Yellow: PAO1 adapted in 1 mM 5-FU



# Ga(NO<sub>3</sub>)<sub>3</sub> synergizes with 5-Fluorocytosine (5-FC) to inhibit *P. aeruginosa* growth and virulence

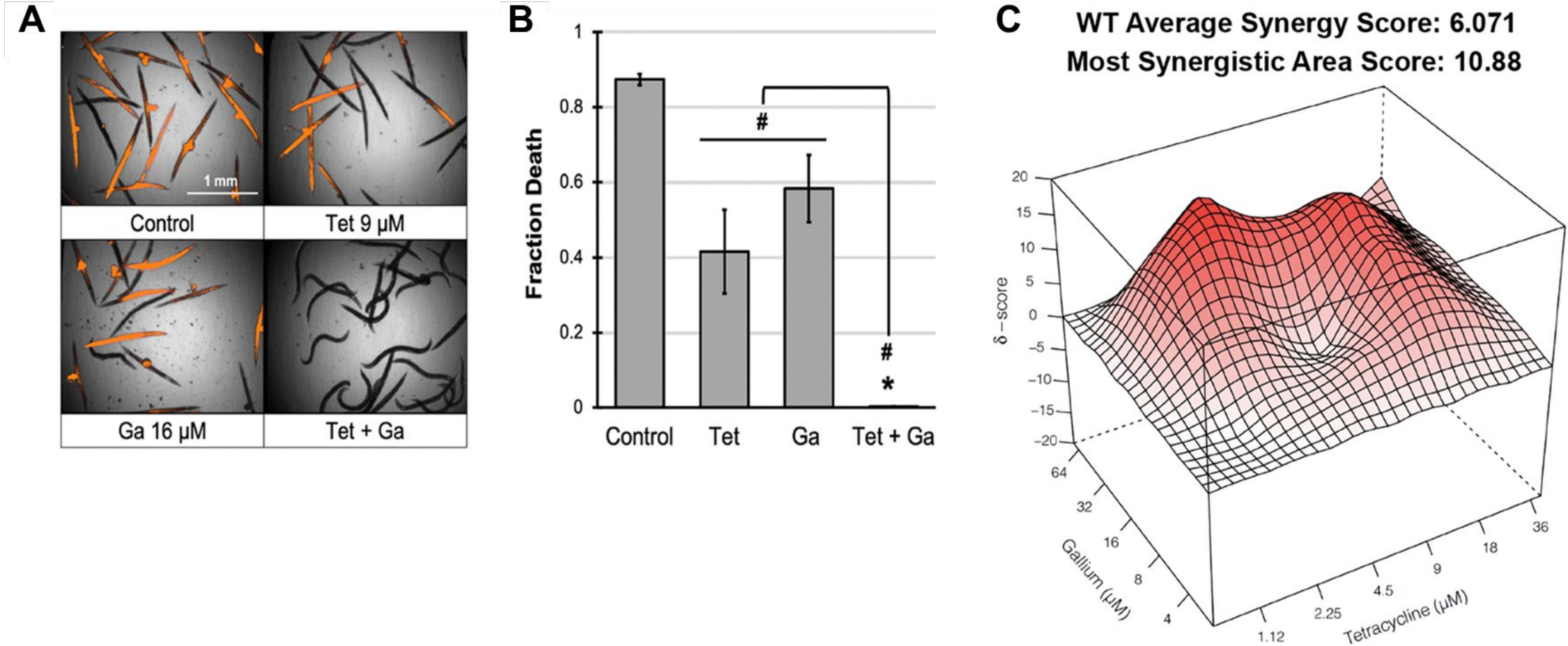


**D**

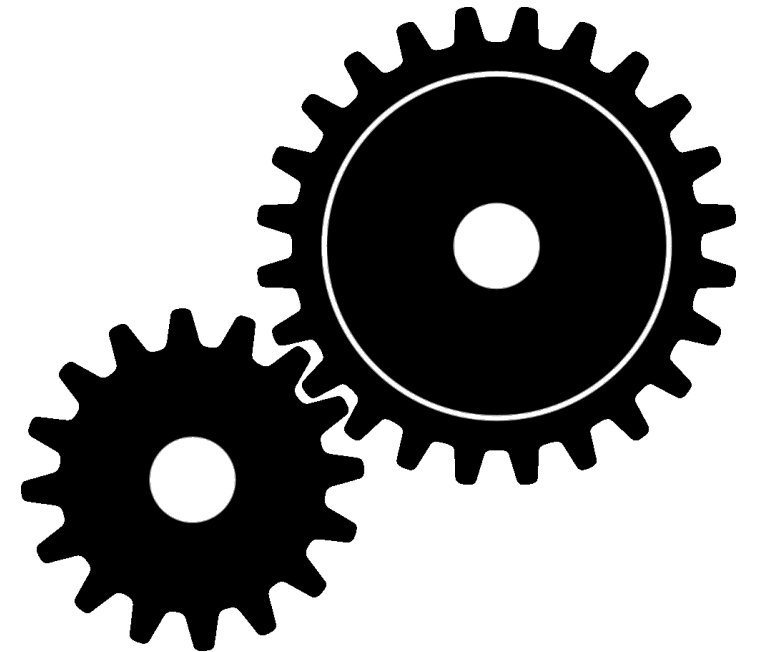
Model	Average Synergy Score	Most Synergistic Area Score
Bliss	11.848	22.76
HSA	11.193	23.55
ZIP	12.061	20.67

Adapted from Kang et. al., (2019)

# Tetracycline synergizes with $\text{Ga}(\text{NO}_3)_3$ to inhibit *P. aeruginosa*



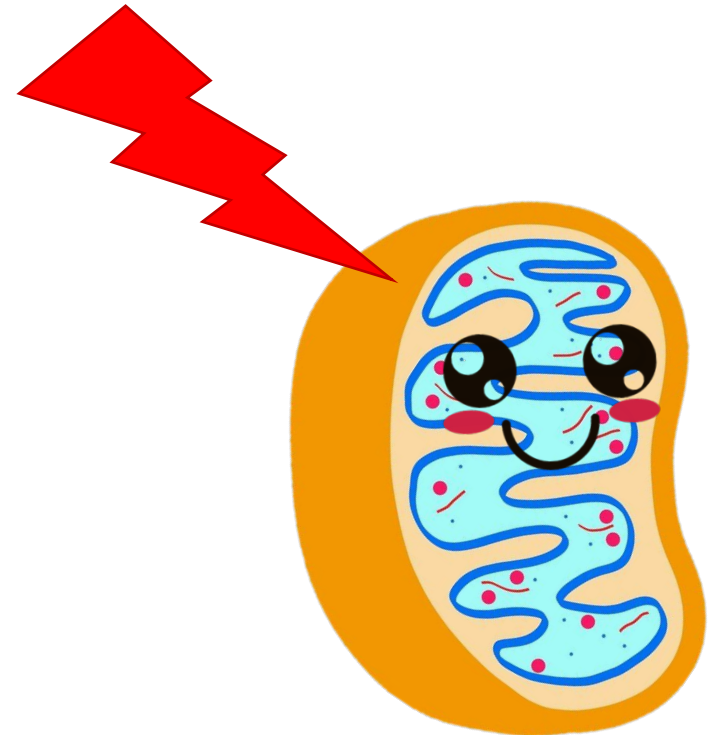
Shifting gears  
now.....



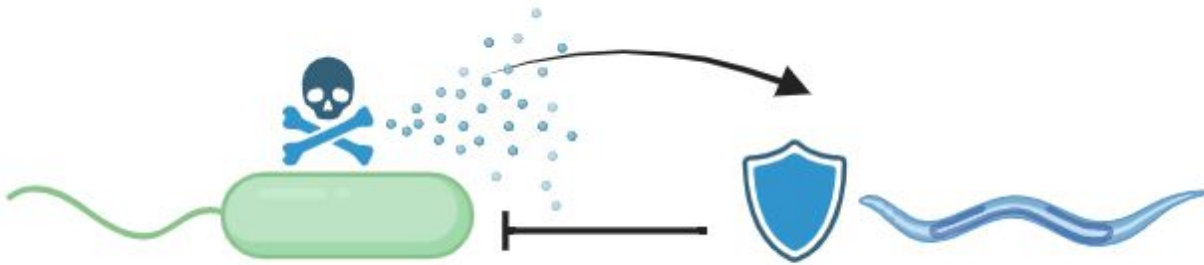


# Mitochondrial function is monitored by surveillance pathways

1. Ethanol and Stress Response Element (ESRE)
2. Mitochondrial Unfolded Protein Response (UPRmt)
3. Mitochondrial MAPK (MAPKmt)
4. PINK1/Parkin (Mitophagy)

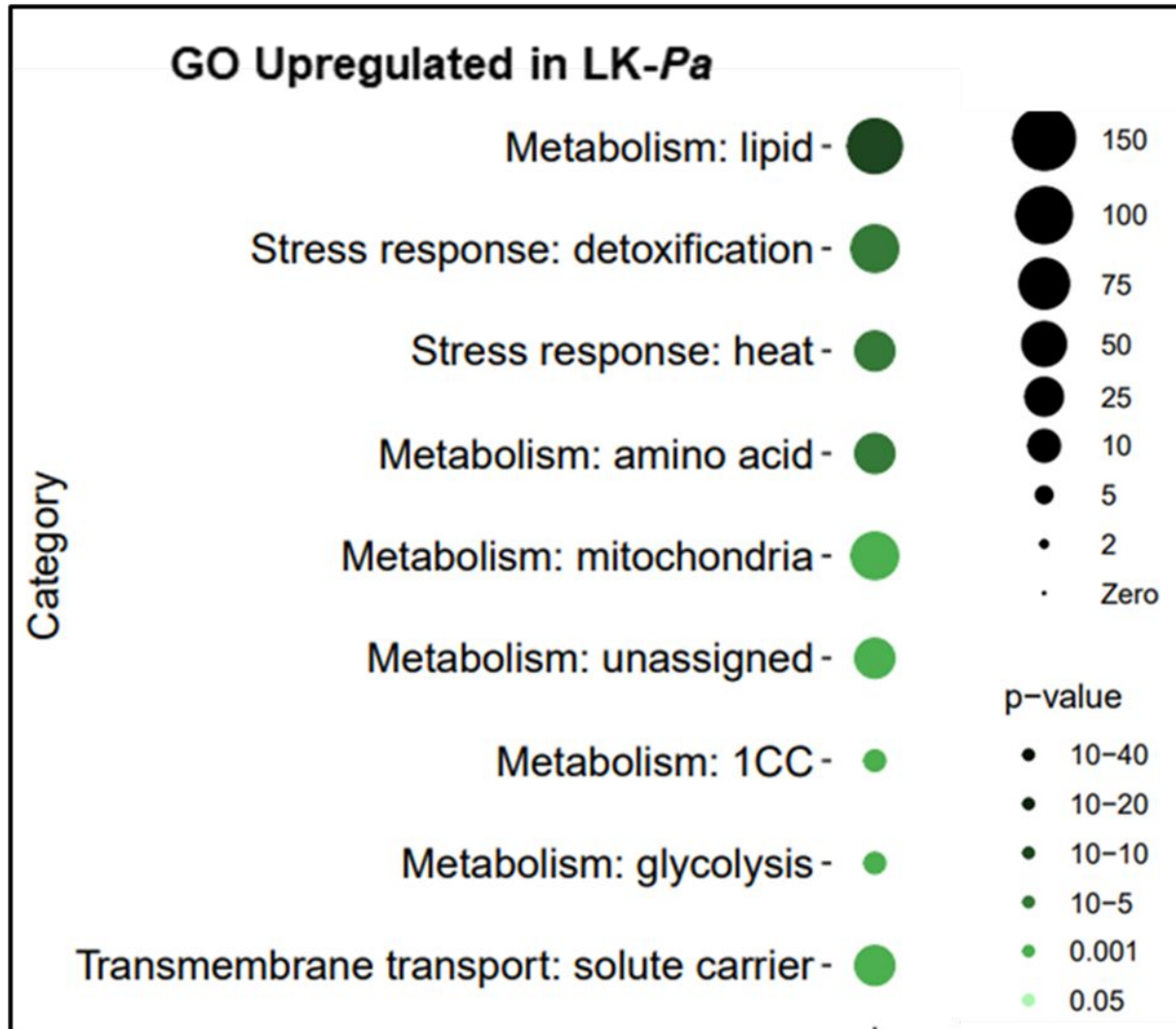


# Abiotic and biotic stresses trigger mitochondrial surveillance pathway activation



- Liquid-based *Pseudomonas aeruginosa* infection damages *C. elegans* mitochondria
- *C. elegans* activates mitochondrial ESRE to mitigate damage
- Mechanism of activation unknown

# Liquid-based infection by *Pseudomonas aeruginosa* (LK-*Pa*) triggers host lipid metabolism changes



- Previous research in *C. elegans* demonstrated involvement of lipids in innate immune response activation
- Could this be the case here?

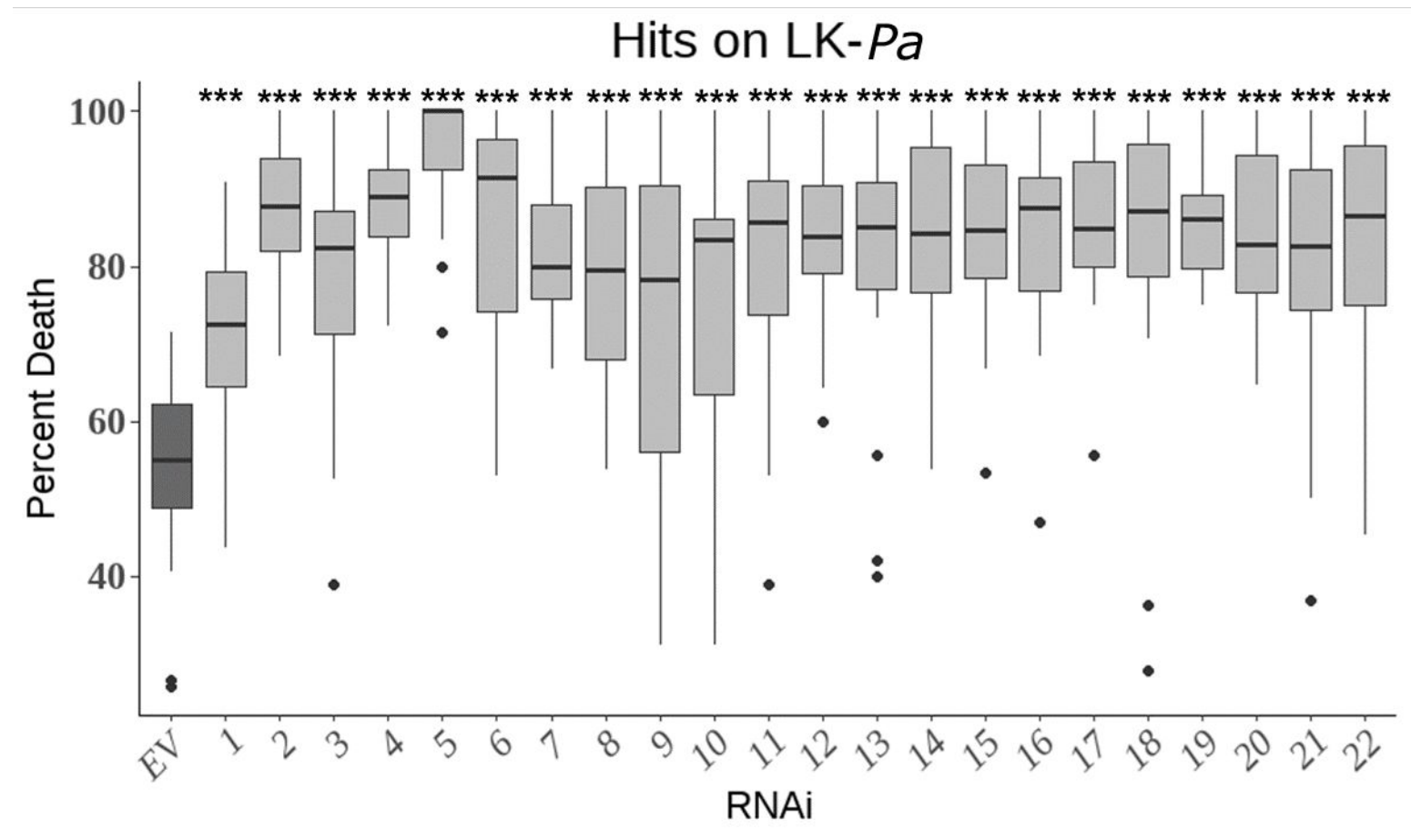
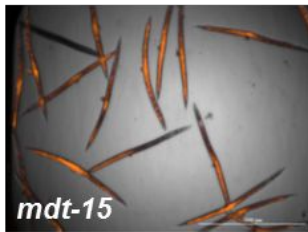


# Lipid metabolism is involved in host defense against LK-*Pa*

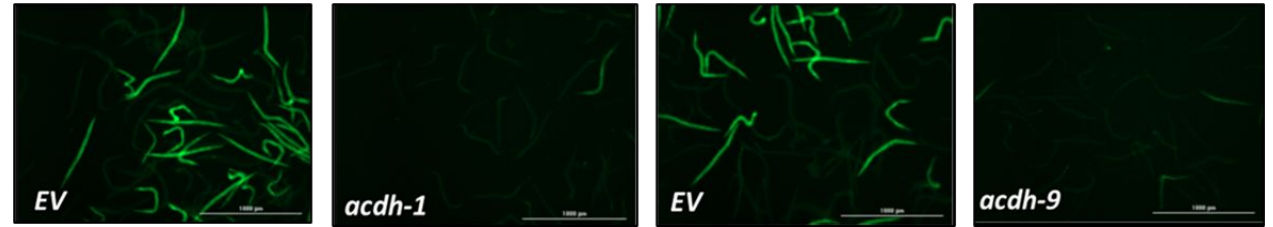
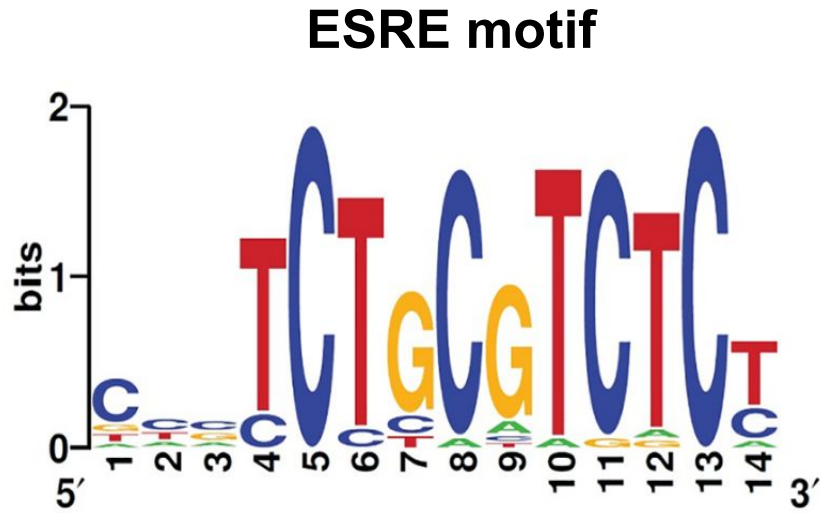
91 lipid metabolism genes upregulated in LK-*Pa*

Primary Screen in LK-*Pa* condition

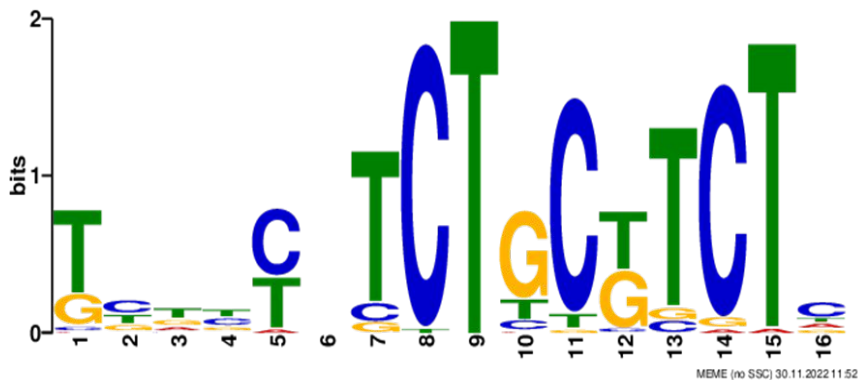
22 hits required for survival in LK-*Pa*



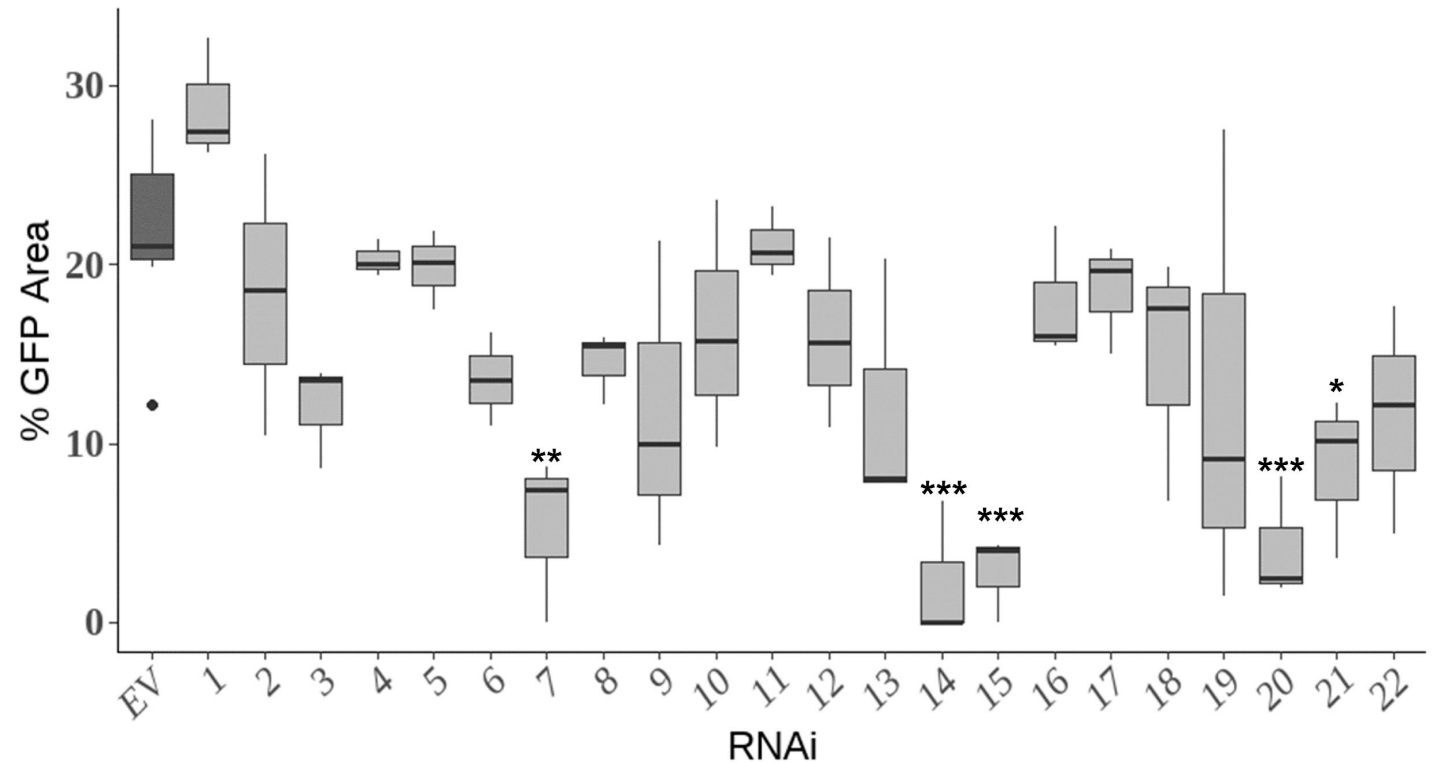
# Lipid metabolism plays a role in ESRE activation



**ESRE Motif is Enriched in the 91 lipid genes Upregulated in LK-*Pa***



**Hits on 3XESRE::GFP**



# Future Directions

1. Determine mechanism of lipid-mediated ESRE activation
2. Test hits on agar-based *P. aeruginosa* infection model (SK-*Pa*)
3. Evaluate if knockdown of our hits causes global lipid changes
4. Supplement hits with different fatty acids to evaluate rescue in *P. aeruginosa* liquid-based infection and ESRE activation



# Acknowledgements

## Kirienko Lab Members

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