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DISCOVERIES FOR HUMANITY

Genetic regulation and innate stress responses in metronidazole resistance in *Giardia*

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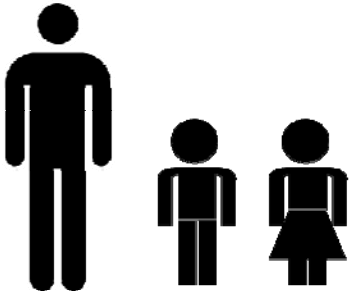
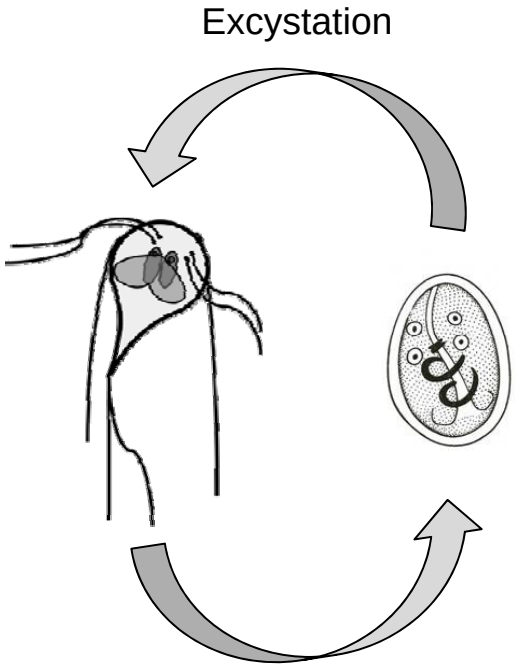
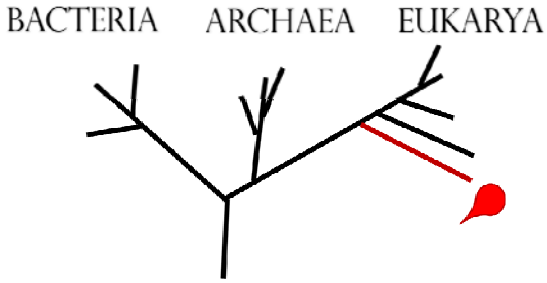
Giardia duodenalis and Giardiasis



- 1 billion infected
- 200-300 million cases
- Children <5 years
- Post-infectious sequelae





- Compact genome
- Amitochondriate
- Bacterial metabolism
- Reduced eukaryote cell biology

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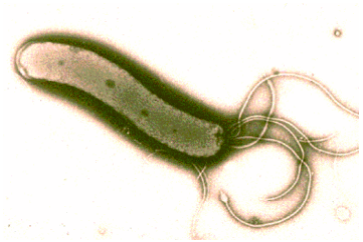
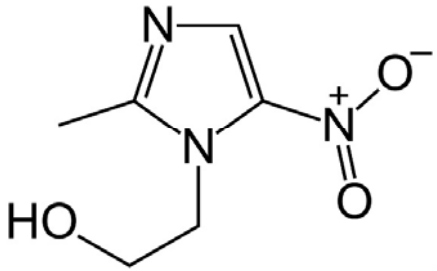
Metronidazole –microaerophilic pathogens



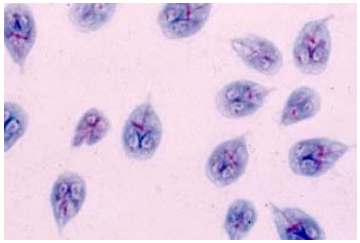
Helicobacter pylori

Bacteriodes fragilis

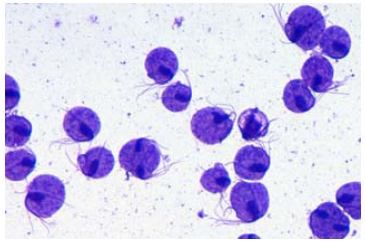
Clostridium sp.



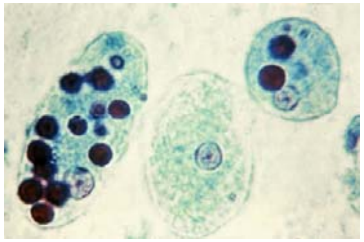
- Nitroheterocyclic
- Pro-drug
- Specific for low dissolved oxygen (reduced)
- Oxidative damage to biomolecules



Giardia duodenalis



Trichomonas vaginalis



Entamoeba histolytica

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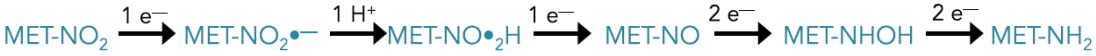
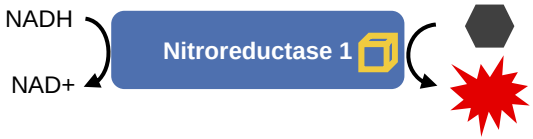
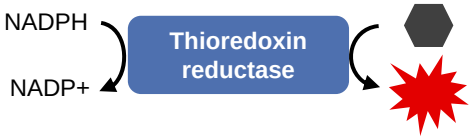
Metronidazole – activation and detoxification



ACTIVATION

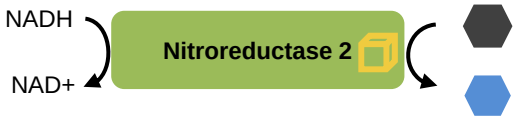
Toxic intermediates

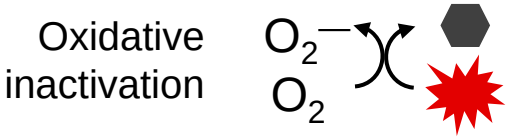




DETOXIFICATION

Inert amine





Ansell et al., Biotechnol Adv., 2015
Leitsch et al., Int J Parasitol 2016

What underpins Mtz resistance?

Differential activity/expression of redox-associated enzymes, and infectivity of laboratory-derived MET-resistant *G. duodenalis*.

Line	Selected resistance	Cross-resistance	Infectivity	MET activation: PFOR		Fd	TrxR	NR-1	MET detoxification: NR-2	NADPH metabolism: NADPH oxidase	GDH	Protein chaperones & HDACs: PDI-2	PDI-4	Sir-2	References
				PFOR-1 17063	PFOR-2 114609	Fd-1 9662	9827	22677	6175	↑	21942	9413	103713	16569	
WB-M3	MET (UV)			a ↓											Townson et al. (1996)
713-M3	MET (UV)	•	0	a ↓		a ↓		t ↓*	t ↑*	a ↓					Leitsch et al. (2011), Liu et al. (2000), Müller et al. (2013), Townson et al. (1996)
WB-C5	MET			t ↓	t ↓			t -	t -		t ↓	t ↑	t -		Müller et al. (2007a), Müller et al. (2013)
1062ID$_{10}$	MET	TIN, ORN	10^5	a ↓		a -	a ↑	t -	t ↑*	a ↓					Leitsch et al. (2011), Müller et al. (2013), Smith et al. (1988), Townson et al. (1996)
WB-M1	MET (UV)	•	0		t ↓*	t ↓*	t -	t ↓*							Tejman-Yarden et al. (2011)
WB-M2	MET (UV)	TIN, ORN	10^6		t -	t ↓*	t -	t ↓*							Tejman-Yarden et al. (2011)
713-M3-C17	MET (UV), C17	TIN, ORN		a ↑		a -		t ↓*	t -	a ↓					Dunn et al. (2010), Leitsch et al. (2011), Müller et al. (2013)
106-C17	C17	MET	0	a -		a -		t ↓*	t -	a ↓					Dunn et al. (2010), Leitsch et al. (2011), Müller et al. (2013)
WB-C4	NTZ	MET		t ↑*	t -	t -	t -	t ↓*	t -	t -	t ↓	t ↑*	t ↑*	t ↓*	Müller et al. (2007a, 2008, 2013), Nillius et al. (2011)
NTZII	NTZ							t ↓*							Nillius et al. (2011)

PFOR Fd TrxR NR1 — NR2

ACTIVATION — DETOXIFICATION

- How does response to Mtz differ from other stress responses?
- Are there multiple pathways to Mtz resistance?
- Are these pathways equal in clinical relevance?
- What regulates these responses?

Ansell et al., Biotech Adv 2015

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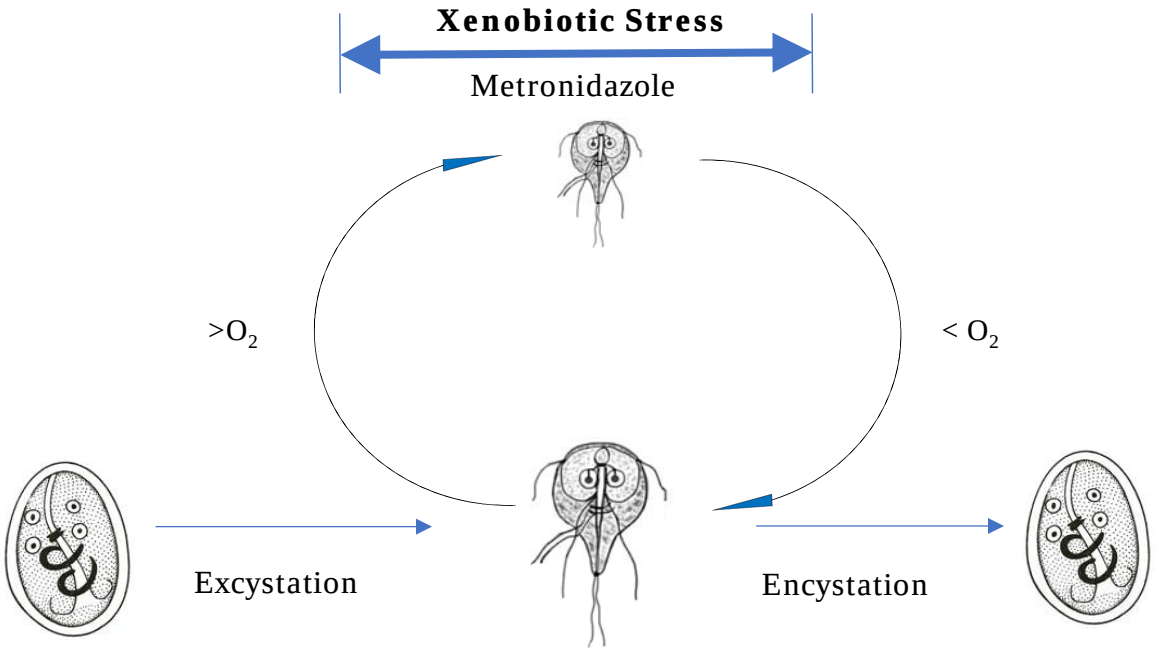
Giardia mounts specific stress responses



- Oxidative burst
- Immune Response
- Increases Temperature (Fever)

Spycher et al;2013 B. Ansell et al; 2015, B. Ansell et al; 2016, B. Ansell et al; 2017

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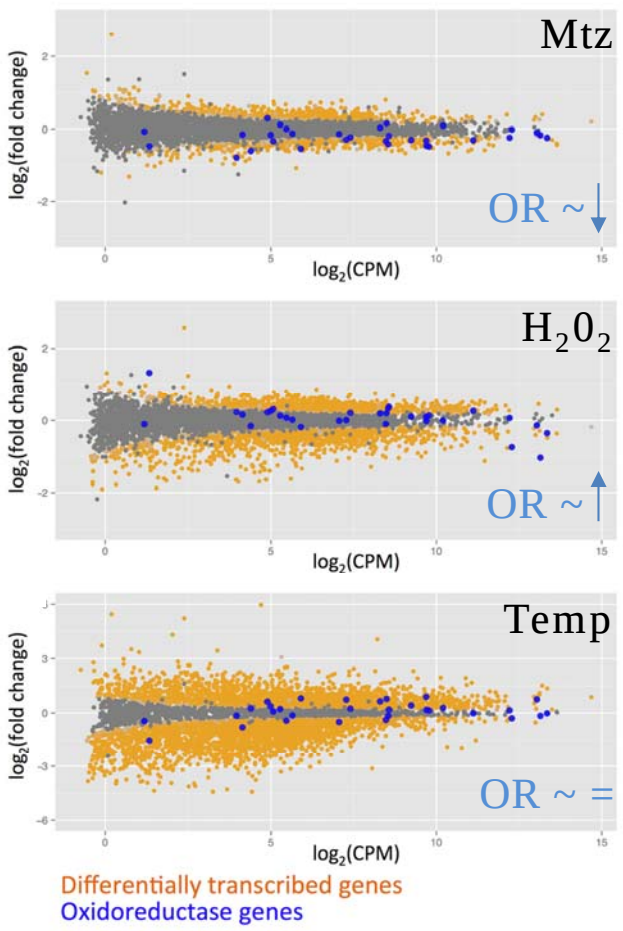
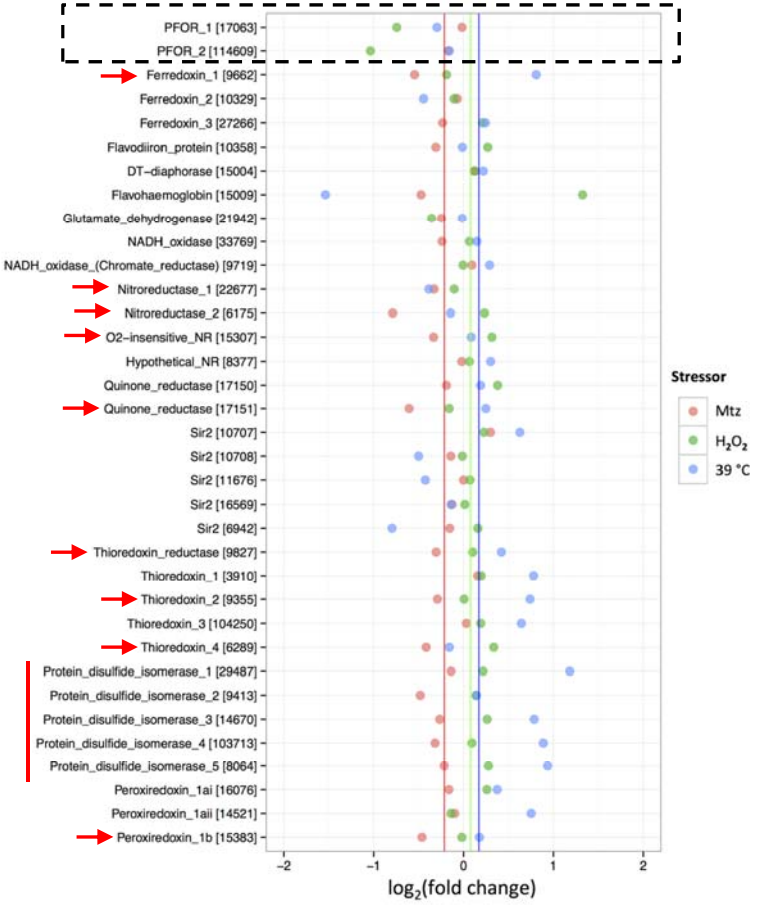
SCOVERIES FOR

Giardia mounts stress specific responses





Ansell et al., AAC 2016

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Are all Giardia Mtz responses equal?

Differential activity/expression of redox-associated enzymes, and infectivity of laboratory-derived MET-resistant *G. duodenalis*.

Line	Selected resistance	Cross-resistance	Infectivity	MET activation					MET detoxification	NADPH metabolism		Protein chaperones & HDACs			References
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WB-C5	MET			t ↓	t ↓			t -	t -		t ↓	t ↑	t -		Müller et al. (2007a), Müller et al. (2013)
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WB-M1	MET (UV)	•	0		t ↓*	t ↓*	t -	t ↓*							Tejman-Yarden et al. (2011)
WB-M2	MET (UV)	TIN, ORN	10^6		t -	t ↓*	t -	t ↓*							Tejman-Yarden et al. (2011)
713-M3-C17	MET (UV), C17	TIN, ORN		a ↑		a -		t ↓*	t -	a ↓					Dunn et al. (2010), Leitsch et al. (2011), Müller et al. (2013)
106-C17	C17	MET	0	a -		a -		t ↓*	t -	a ↓					Dunn et al. (2010), Leitsch et al. (2011), Müller et al. (2013)
WB-C4	NTZ	MET		t ↑*	t -	t -	t -	t ↓*	t -	t -	t ↓	t ↑*	t ↑*	t ↓*	Müller et al. (2007a, 2008, 2013), Nillius et al. (2011)
NTZII	NTZ							t ↓*							Nillius et al. (2011)

PFOR Fd TrxR NR1 NR2

ACTIVATION DETOXIFICATION

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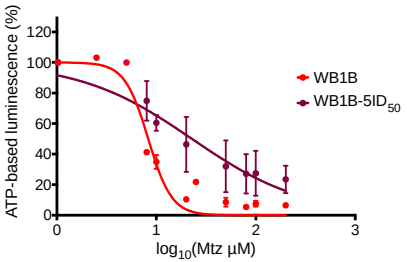
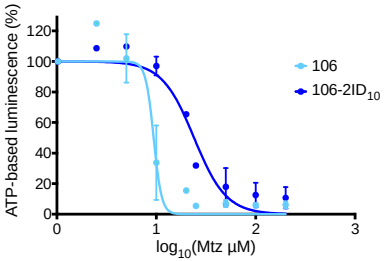
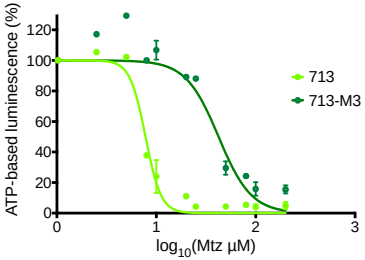
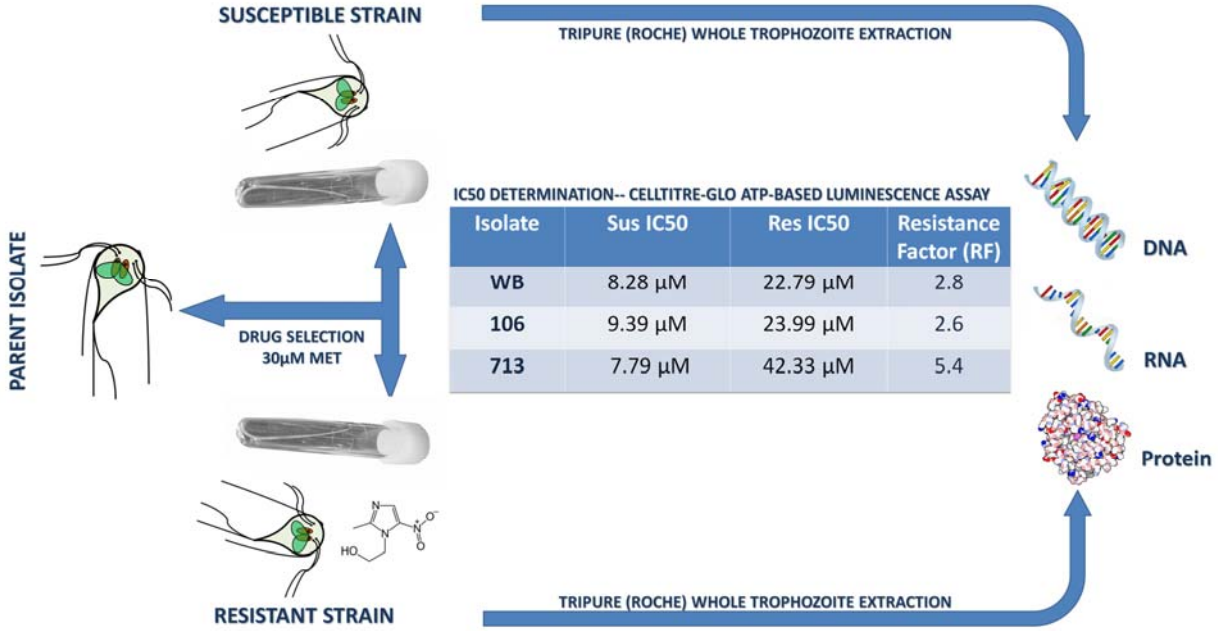
3-way isogenic isolate (Mtz-R vs Mtz-S) analysis









IC50 DETERMINATION-- CELLTITRE-GLO ATP-BASED LUMINESCENCE ASSAY

Isolate	Sus IC50	Res IC50	Resistance Factor (RF)
WB	8.28 µM	22.79 µM	2.8
106	9.39 µM	23.99 µM	2.6
713	7.79 µM	42.33 µM	5.4

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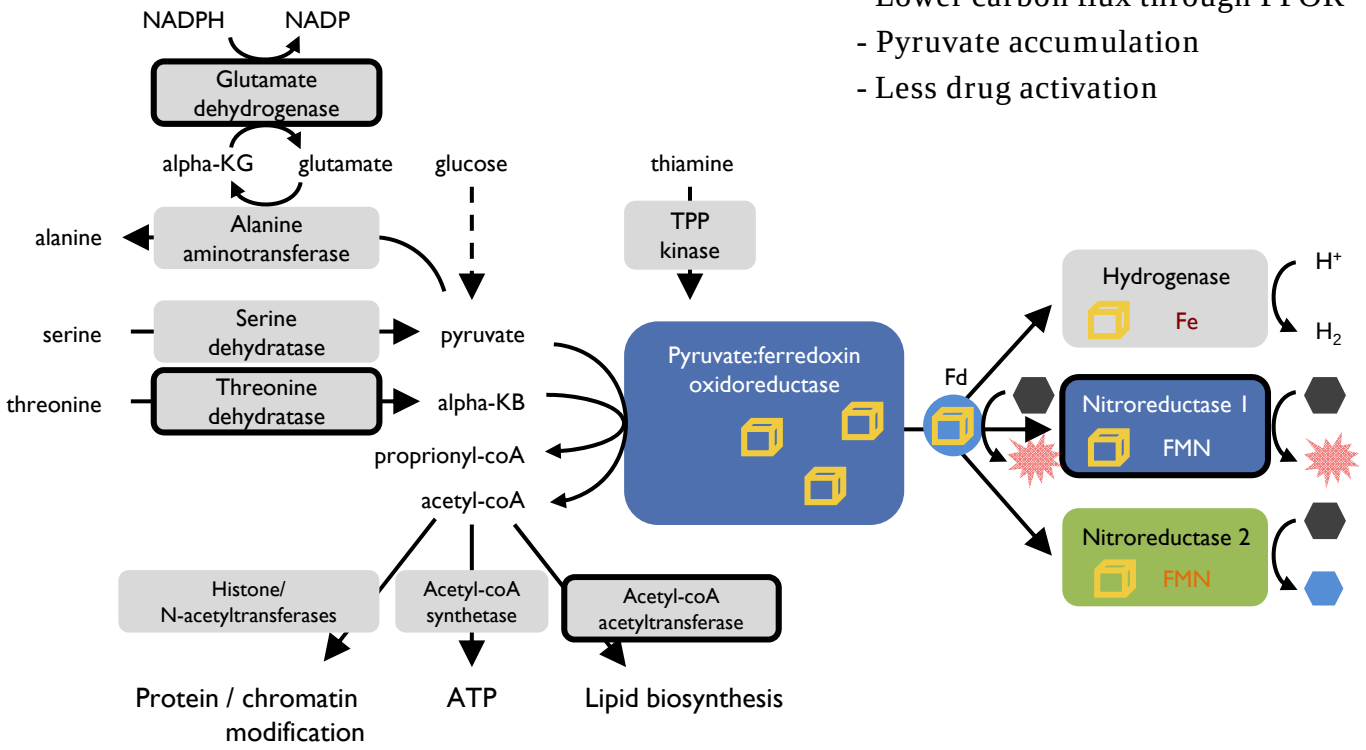
Resistant vs susceptible — common genes

- Lower carbon flux through PFOR
- Pyruvate accumulation
- Less drug activation



Ansell et al., Frontiers Microbiol 2017

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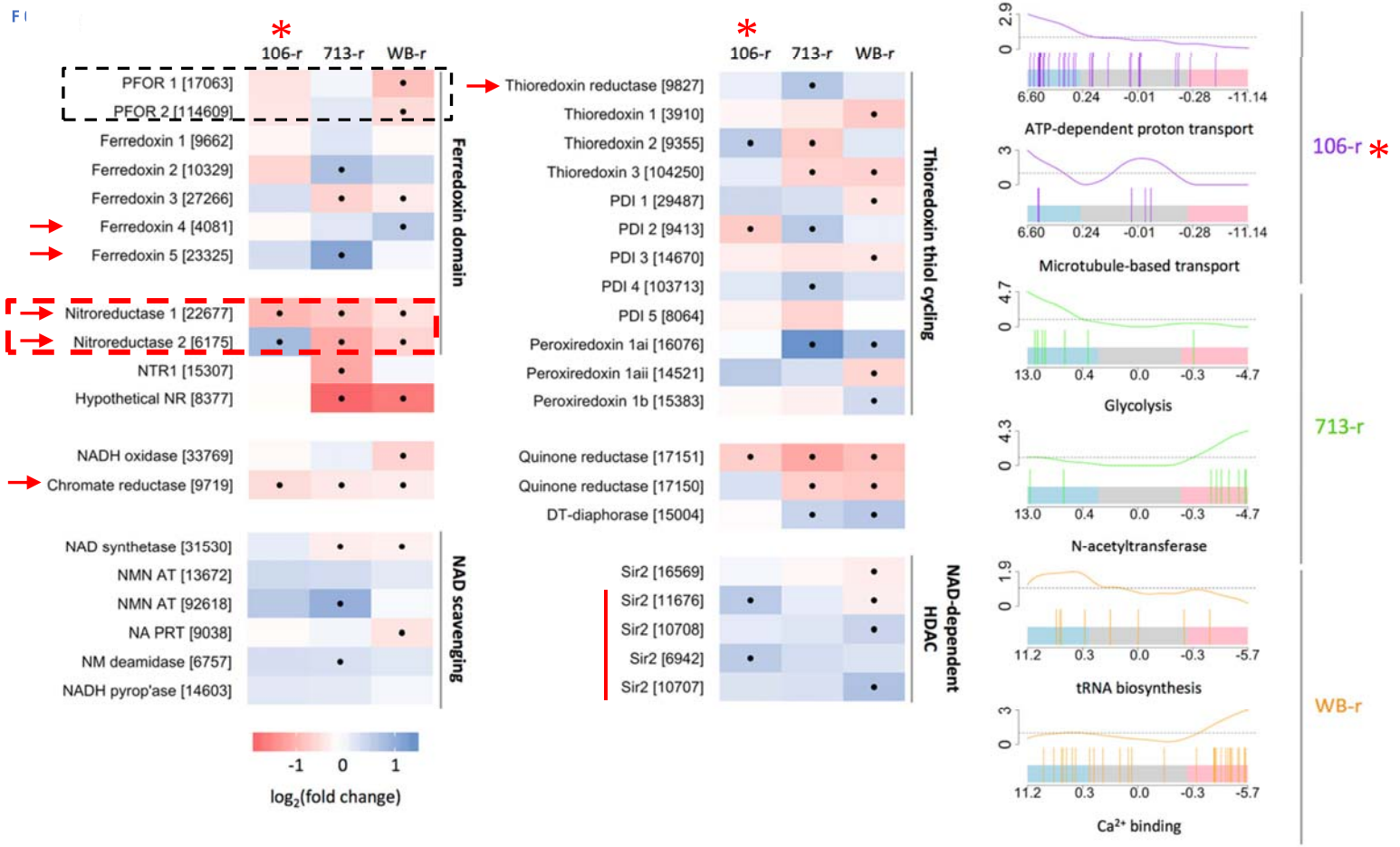
Isotype-specific transcriptional changes



Ansell et al., Frontiers Microbiol 2017

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What regulates Mtz resistance?

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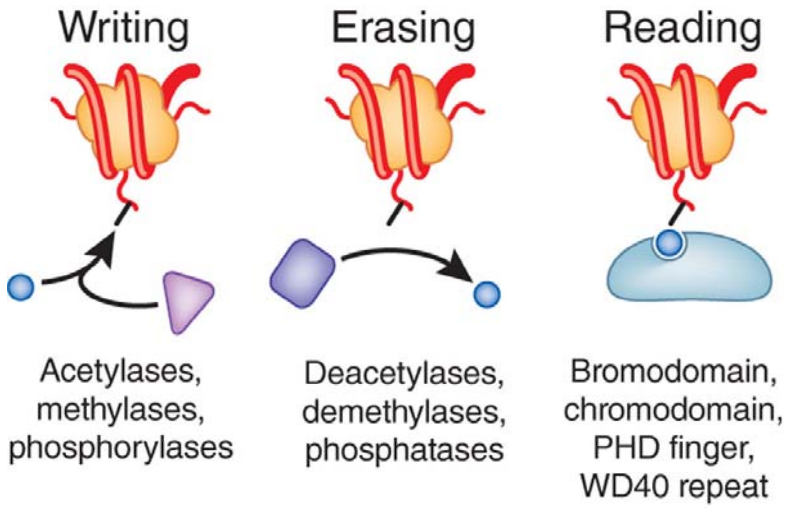
Role of post-translation modifications in Mtz-R

- Post-transcriptional regulation
  - Protein post-translational modifications
- MtzR is an unstable/plastic phenotype.
  - Ongoing drug selection
  - Reset during differentiation
- Acetylation in MtzR
  - NAD+-dependent Sirtuins
  - *Muller et al, 2008*
  - *Ansell et al, 2015*



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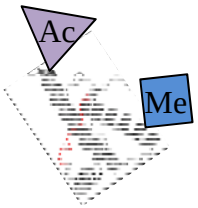
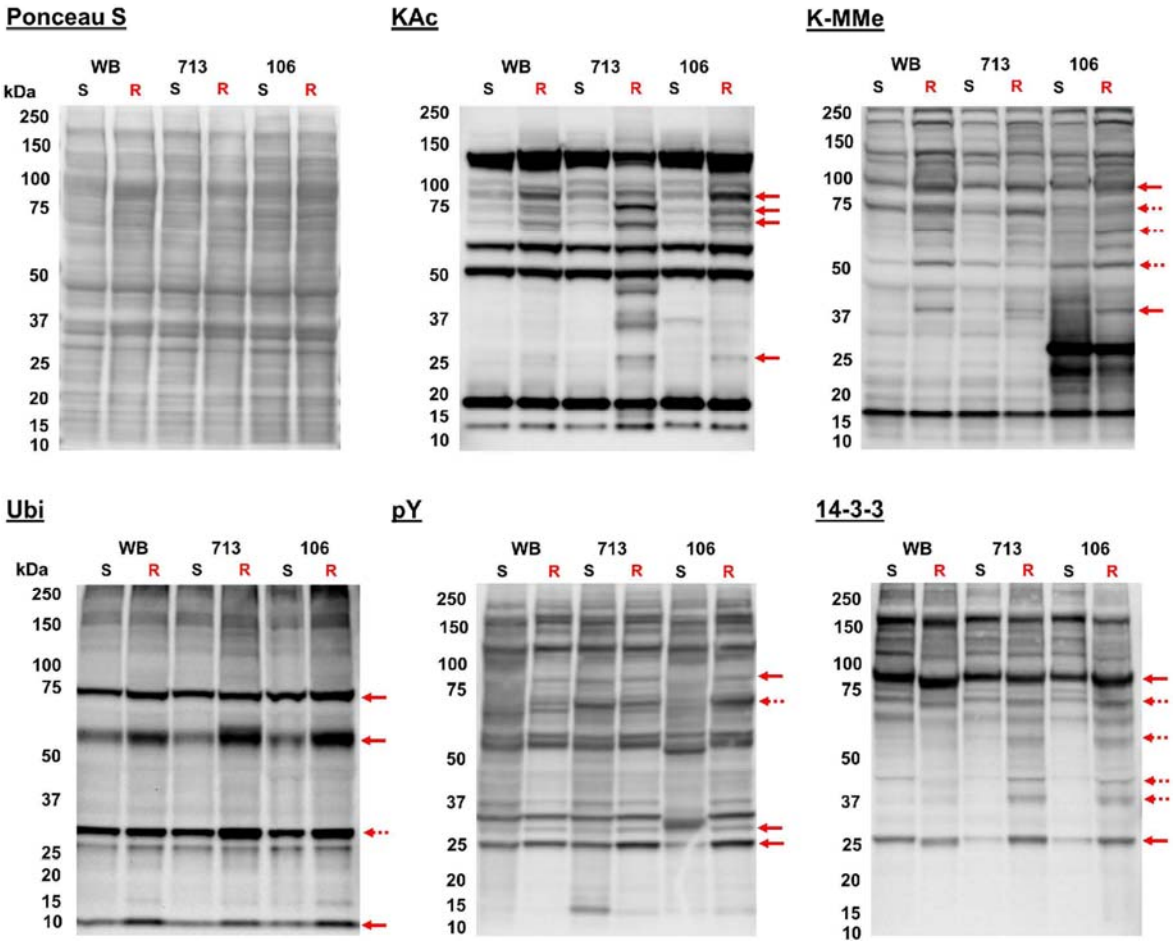
Shift in PTM profiles with Mtz-R among all lines



Lysine Acetylation
Lysine Methylation

Serine/Threonine & Tyrosine Phosphorylation



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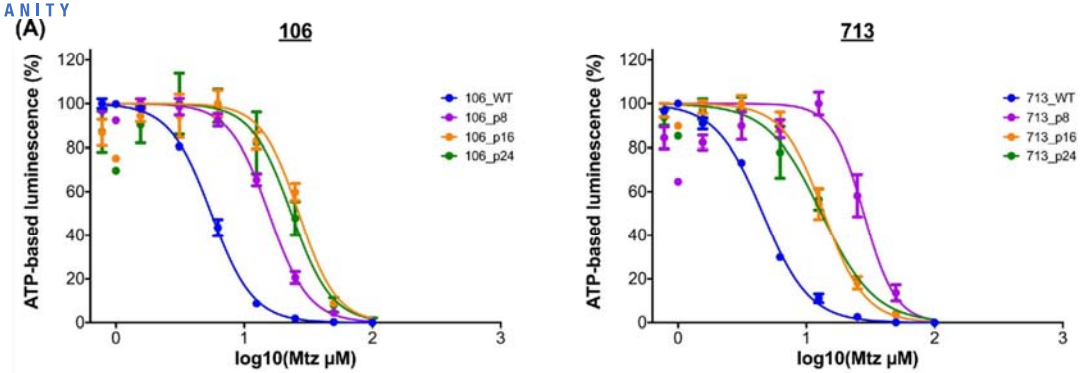
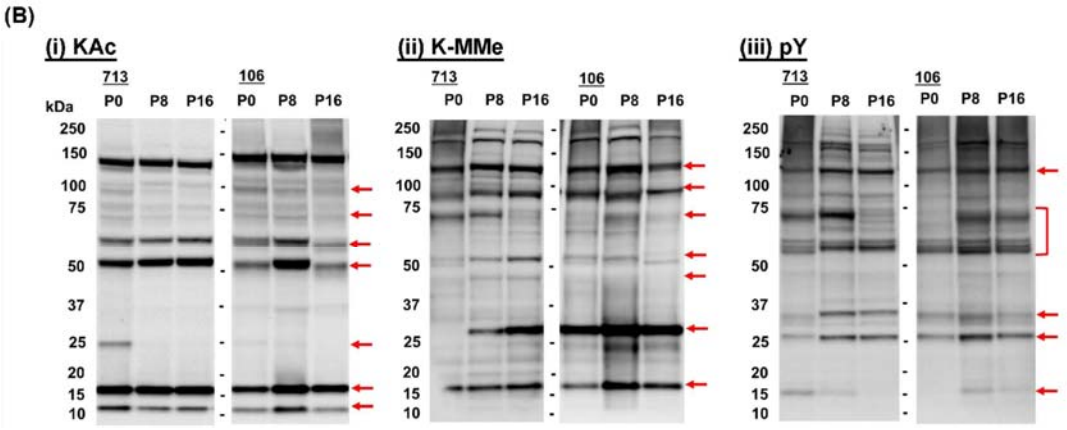
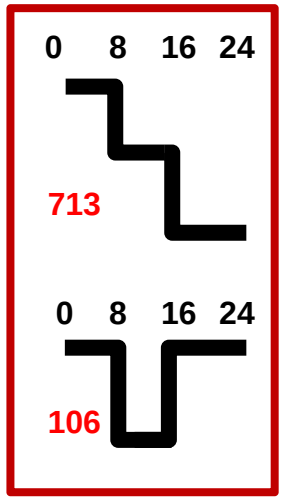
Are these changes stable with in vitro passage?



	WT	P8		P16		P24	
	Mtz IC_{50} (µM)	Mtz IC_{50} (µM)	RF	Mtz IC_{50} (µM)	RF	Mtz IC_{50} (µM)	RF
106	5.5	15.6	2.8	26.7	4.8	23.4	4.2
713	4.6	28.1	6.1	13.7	3.0	13.1	2.9





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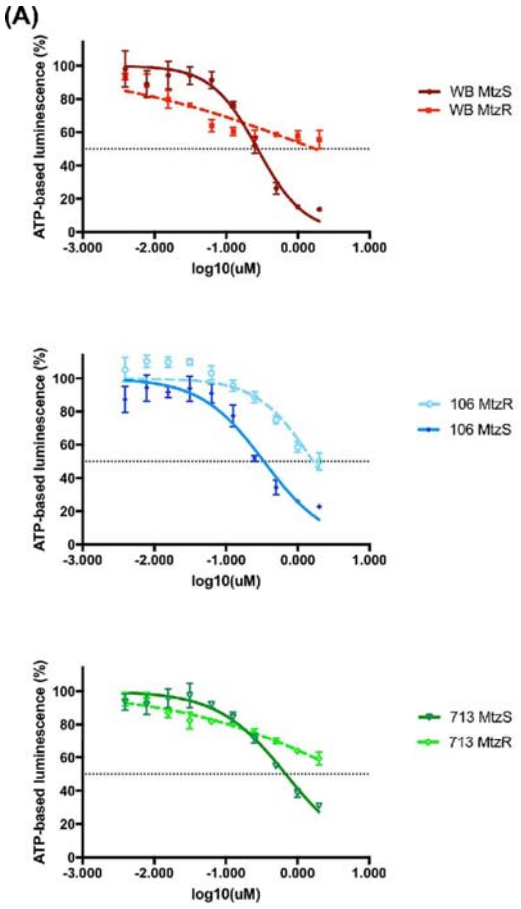
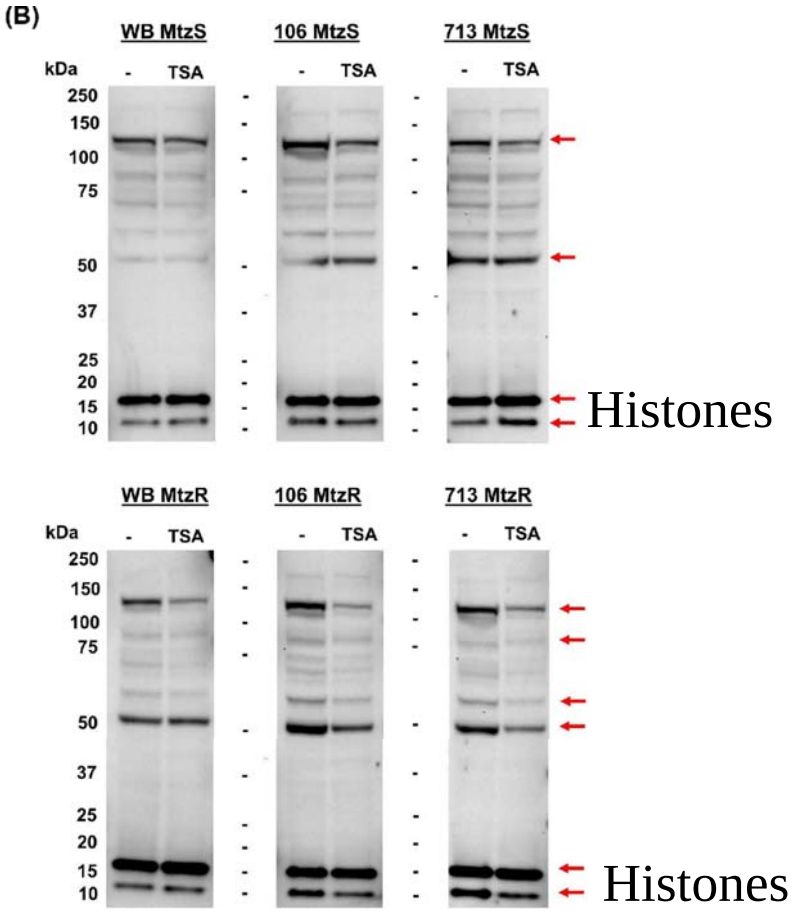
Trichostatin influences Mtz effect





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Summary and Future Research

- MtzR complex – Multiple pathways to resistance
- Many *in vitro* MtzR impart loss of fitness / reduced growth
- 106 MtzR ‘stable’ / more focused
- NR1/NR2 reconfiguration a core change
- Other consistent changes in Sir2s, TrX, Ferrodoxin,
- PFOR1/PFOR2 – maybe not as important as previously thought?
- PTM involvement – Histone Lysine Acetylation is Mtz-R?

Future work

- Explore dose-dependent transcriptomic changes – Mtz ‘Road to Resistance’
- Characterize PTM regulation (S. Emery-Corbin) and other ”epigenetic” mechanisms
- Examine consistent changes and contrast ‘stable’ (e.g., 106) vs ‘unstable’ Mtz-R
- Role for post-transcriptional regulation (sRNAs/RNA-binding proteins) in Mtz-R

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  - Showgy Ma'ayeh
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  - Andrew Webb
- **YourGene Biosciences**
  - Bill Chang

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