Expression of human H ferritin prompts the identification of a hitherto elusive yeast orthologue and enables parsing of distinct iron-induced cell death pathways in *Saccharomyces cerevisiae.*

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11th IMYA

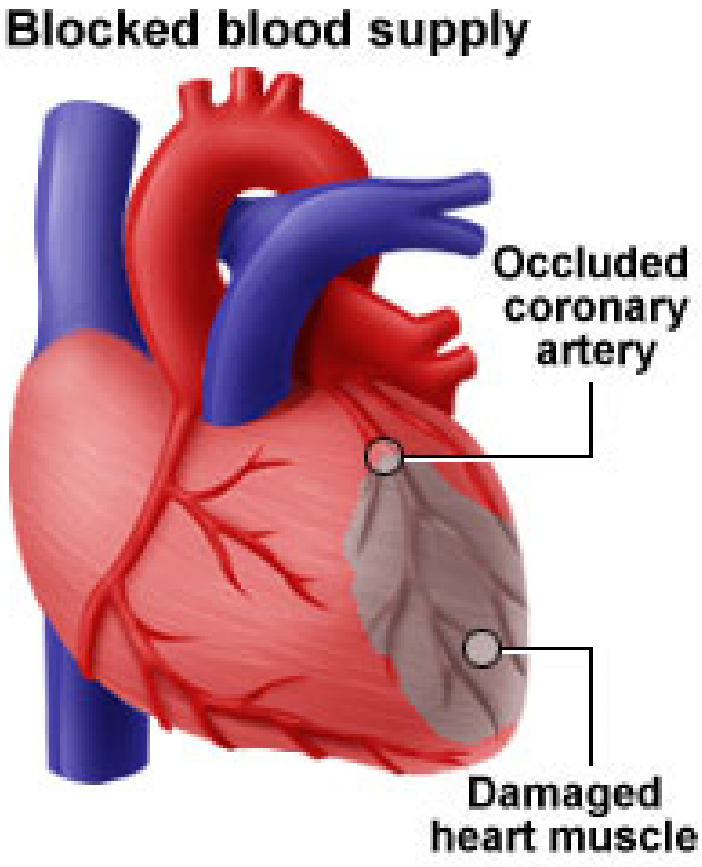
September 29th – October 3rd 2015

Porto, Portugal

Acute Cardiac Ischemia leads to necrosis and apoptosis

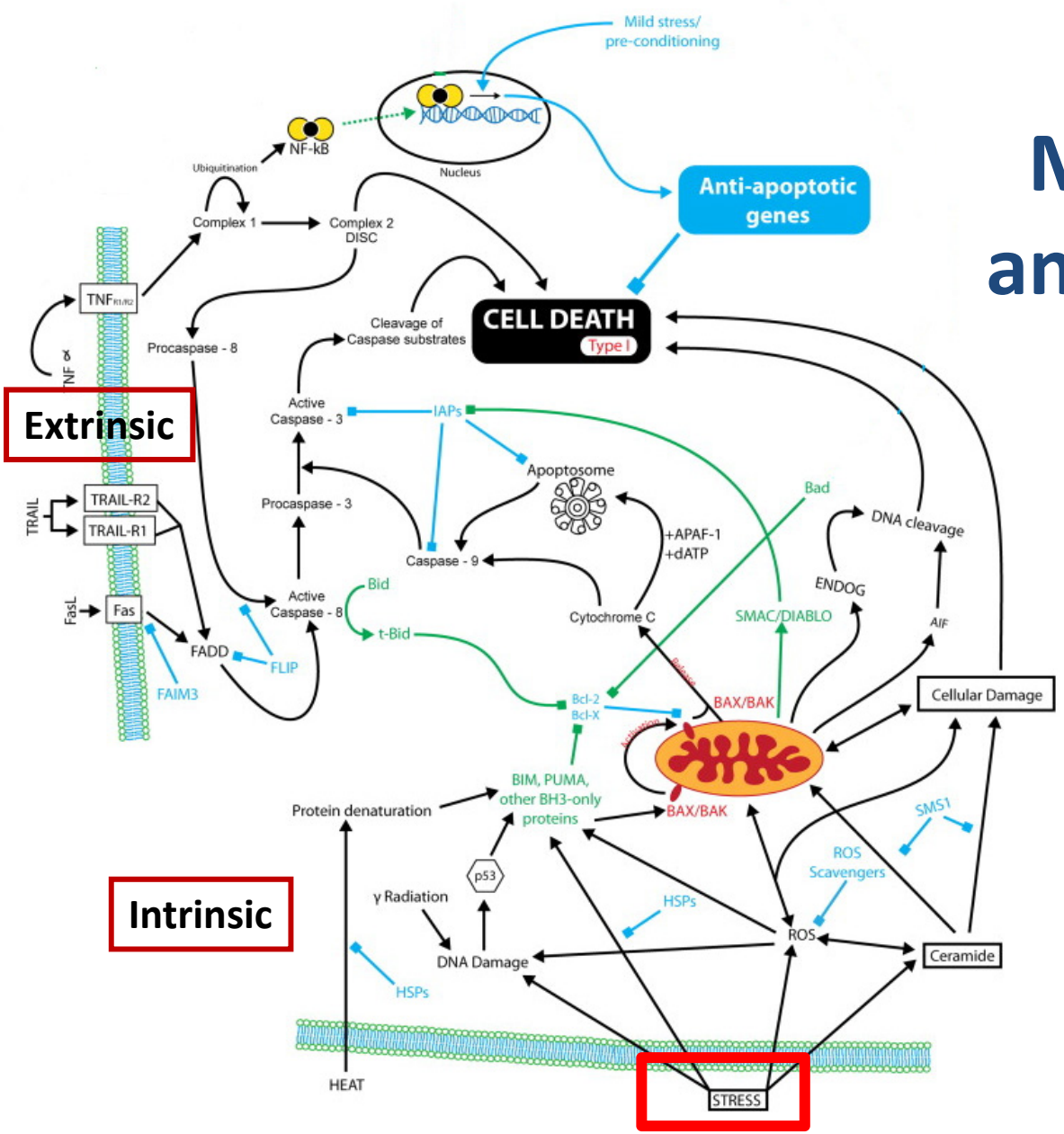
- Blockage in the coronary arterioles cuts off blood supply to downstream tissue
- The most centrally located area of the halted blood flow has the highest degree of ischemia
- Ischemia and subsequent reperfusion of the tissue leads to apoptosis.
- **Delivery of anti-apoptotic genes would likely have very potent therapeutic effects following an ischemia reperfusion event**
- **Proof of principal in the literature:**

Over expression of anti-apoptotic genes decreases cardiac apoptosis (Matsushima et al. 2006) Circulation 113;1779; Fan et al. 2005 Circulation 111;1792)



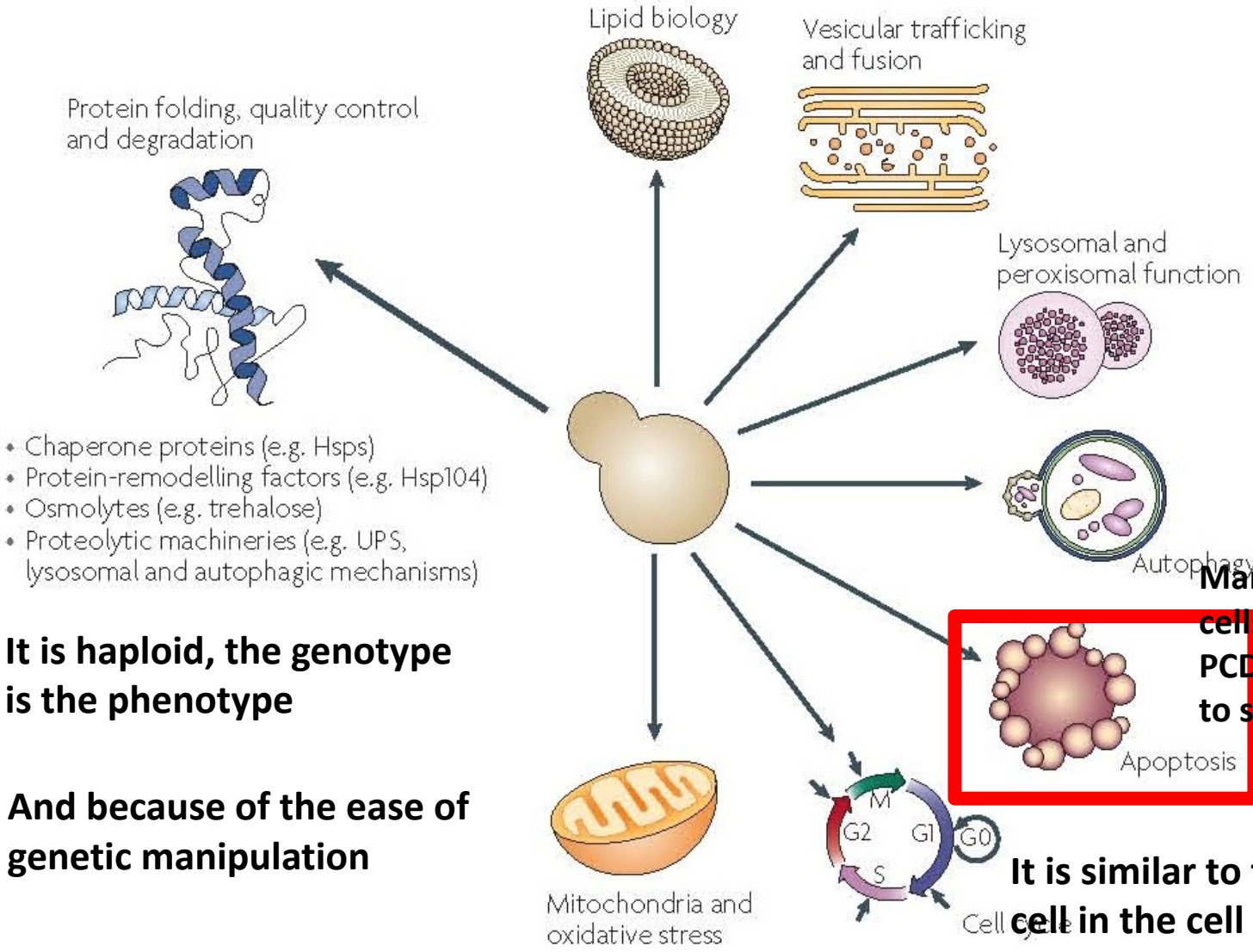
http://pbm.tnw.utwente.nl/people/phd/bat.doc/bat-2.jpg

Complexity of Mammalian Pro- and Anti-Apoptotic Pathways



Portt et al. (2011) Anti-apoptosis and cell survival: A review. BBA 1813:315-321

Yeast Is a Model Eukaryotic Cell…



It is haploid, the genotype is the phenotype

And because of the ease of genetic manipulation

Mammalian and yeast cells both use conserved PCD pathway in response to stress

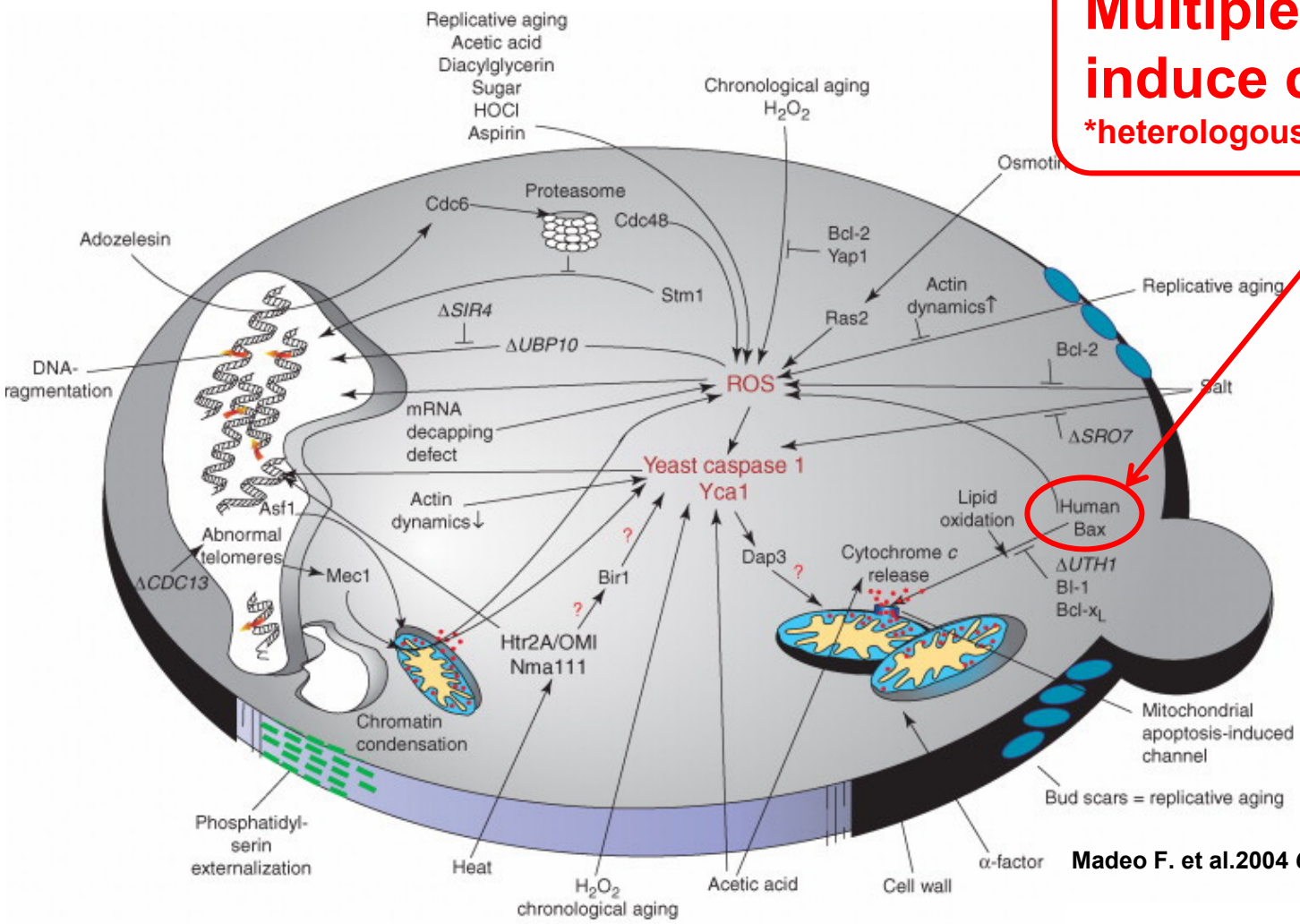
It is similar to the mammalian cell in the cell cycle

Khurana and Lindquist 2010. Modelling neurodegeneration in *Saccharomyces cerevisiae*: why cook with baker's yeast? Nature Reviews Neuroscience

Yeast Apoptosis Pathways Resemble Their Mammalian Counterparts

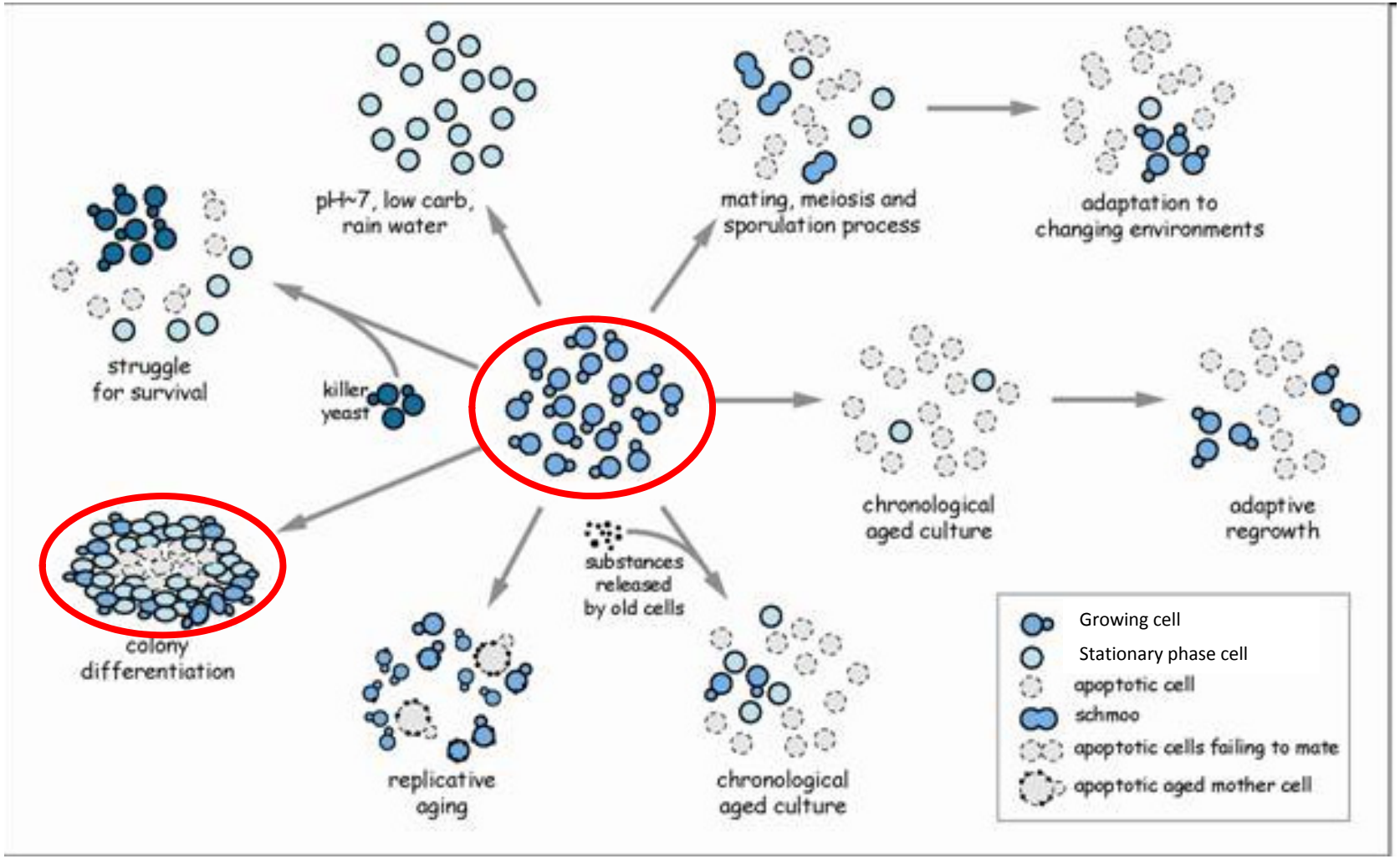
Multiple stresses induce cell death

***heterologously expressed human Bax**



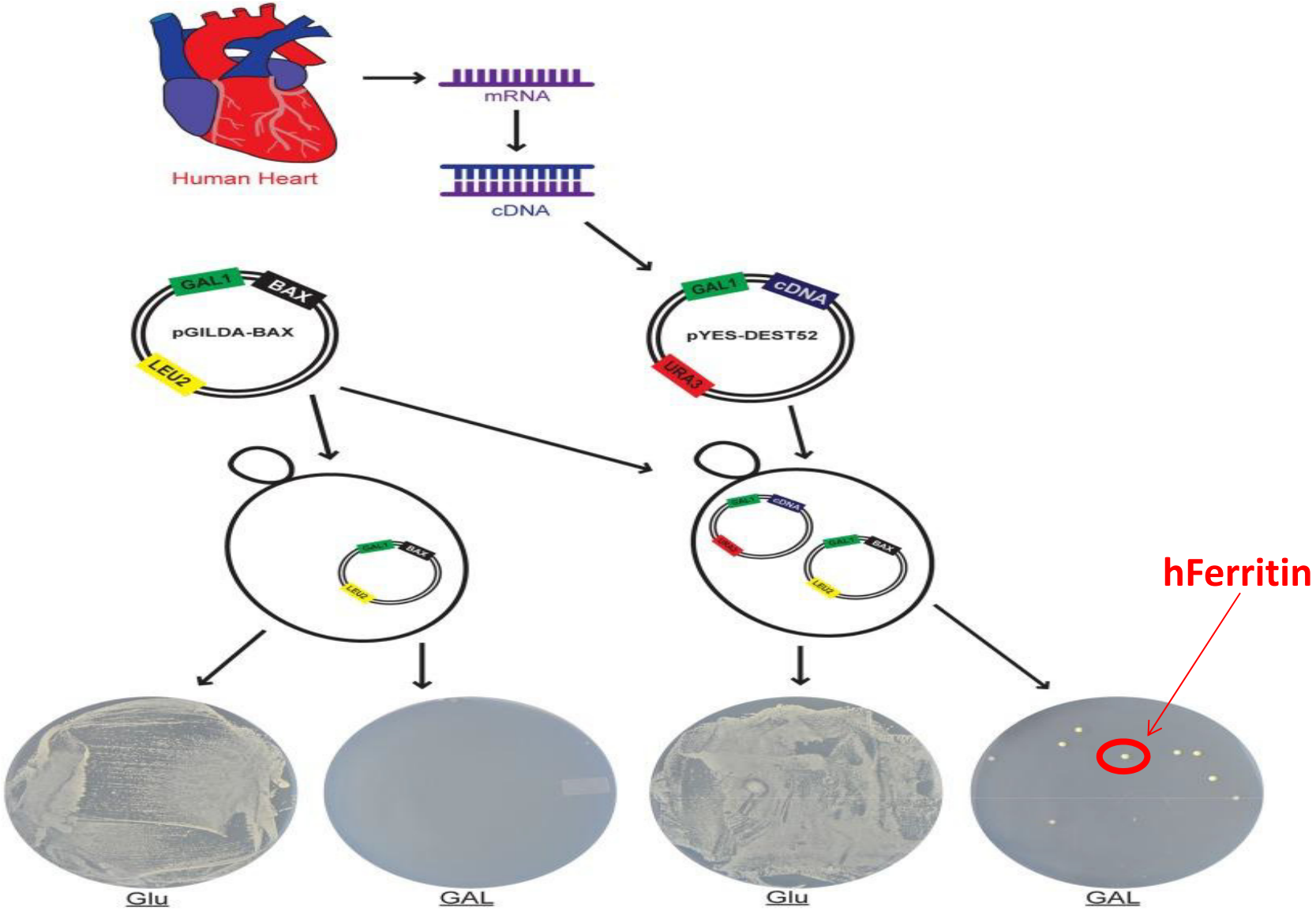
Madeo F. et al.2004 *Curr. Opin. in Microbio.* 7: 655-660.

Programmed cell death in unicellular organism promotes the survival of daughter cells (yeast colonies are clonogenic)



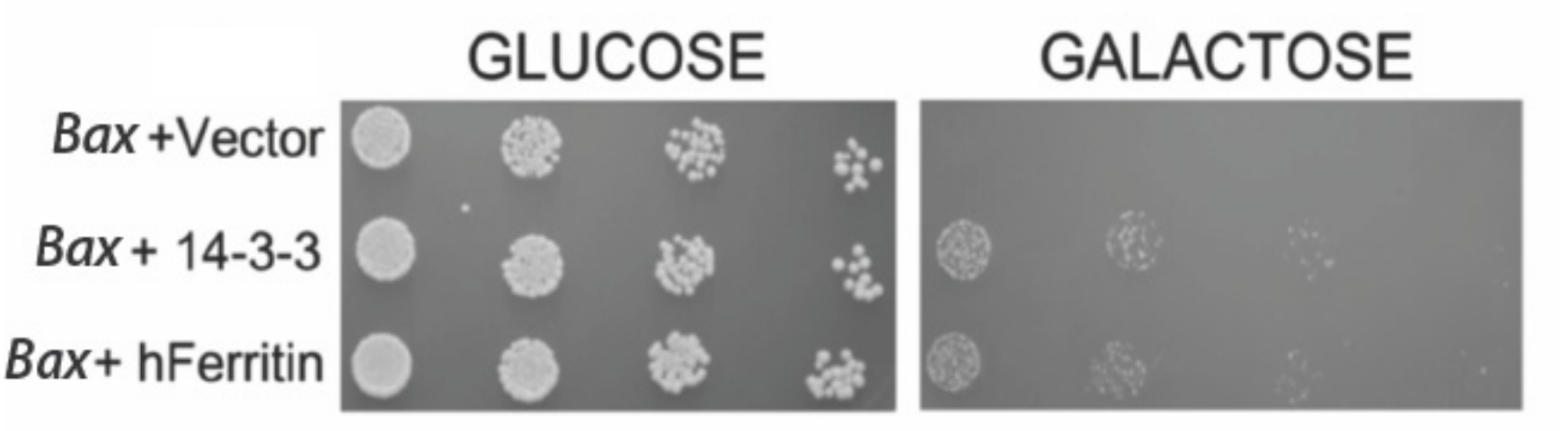
Büttner et al. 2006 J Cell Biol 175:521

Screening for Bax suppressors



Clapp et al 2012 Untangling the roles of anti-apoptosis in regulating programmed cell death using humanized yeast cells

Human Ferritin is a Bax Suppressor



This confirms the results of the screen showing that hFerr is a Bax suppressor and given that ferritin is a known pro-survival protein, hFerr thus appears to be a functional protein in yeast

Ferritin – Like Super Family

12 –Subfamilies

11 –Subfamilies

Ferritin Sub-family

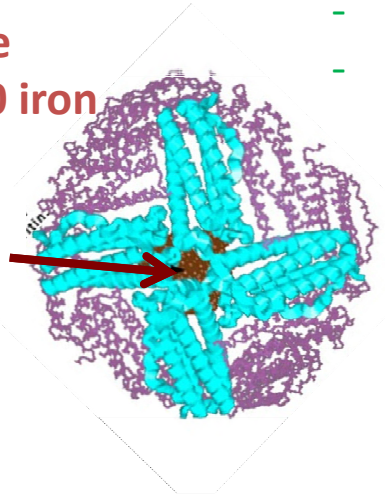
Ferritin

Bacterioferritins

DPS (DNA- binding protein from starved cells)

- Capable of acting as storage molecules.
- Have 24 Subunit structure
- Can store as many as 4000 iron atoms

Iron stored as mineral in ferritin



- Capable of Binding Iron
- Can protect cells from the toxic effects of iron and other free radical producing stresses such as H_2O_2

Metabolism of Iron and the role of Ferritin

Although Iron is very abundant, most forms are insoluble and are thus not easily accessible biologically

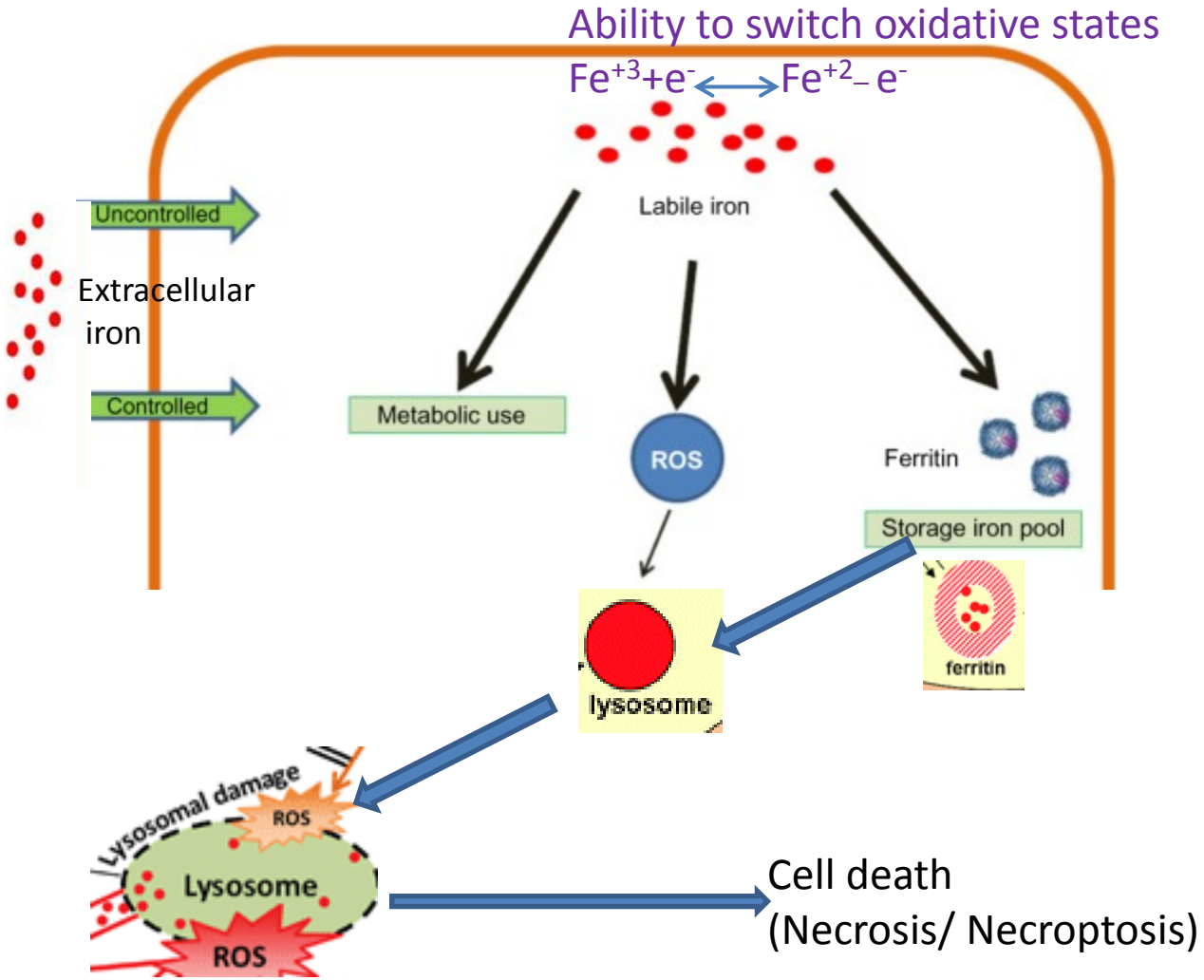
Iron is toxic at high levels

Fenton Reaction

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$

$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\bullet + H^+$

Specific regulatory mechanisms have evolved to allow cells to grow and survive in environments with either excess or limiting levels of iron {Bleackley, 2011}.



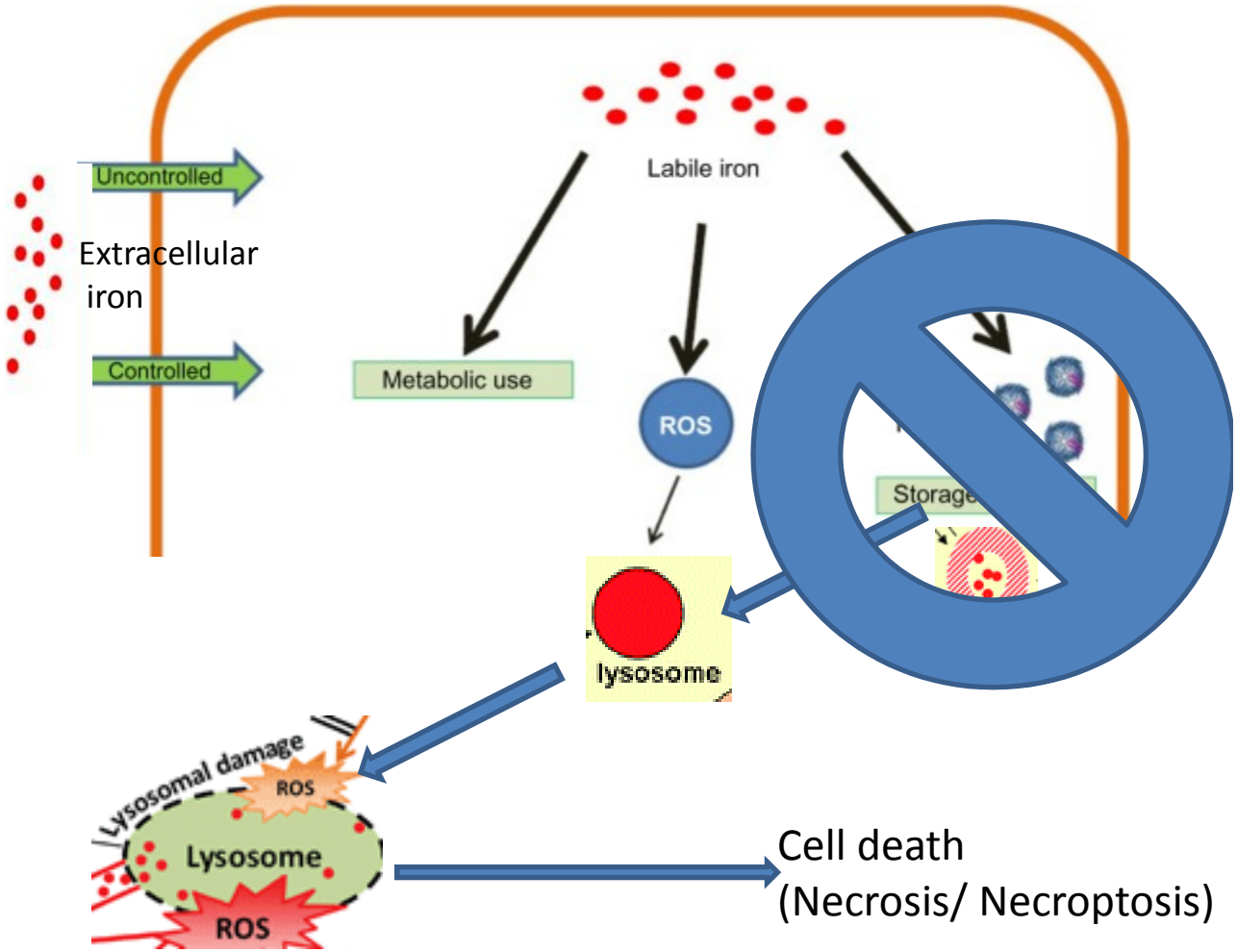
Adapted from BA et al. 2009 Metallomics **1**, 292-311; Aroun et al 2013Photochemical & Photobiological Sciences 11, 118-134; Saliba et al. 2015 J Blood Med. 6: 197–209.

Yeast and Ferritin

•The yeast *S. cerevisiae* is an excellent model to study iron metabolism given that there are a great number of similarities between yeast and humans (Bleackley, 2011}

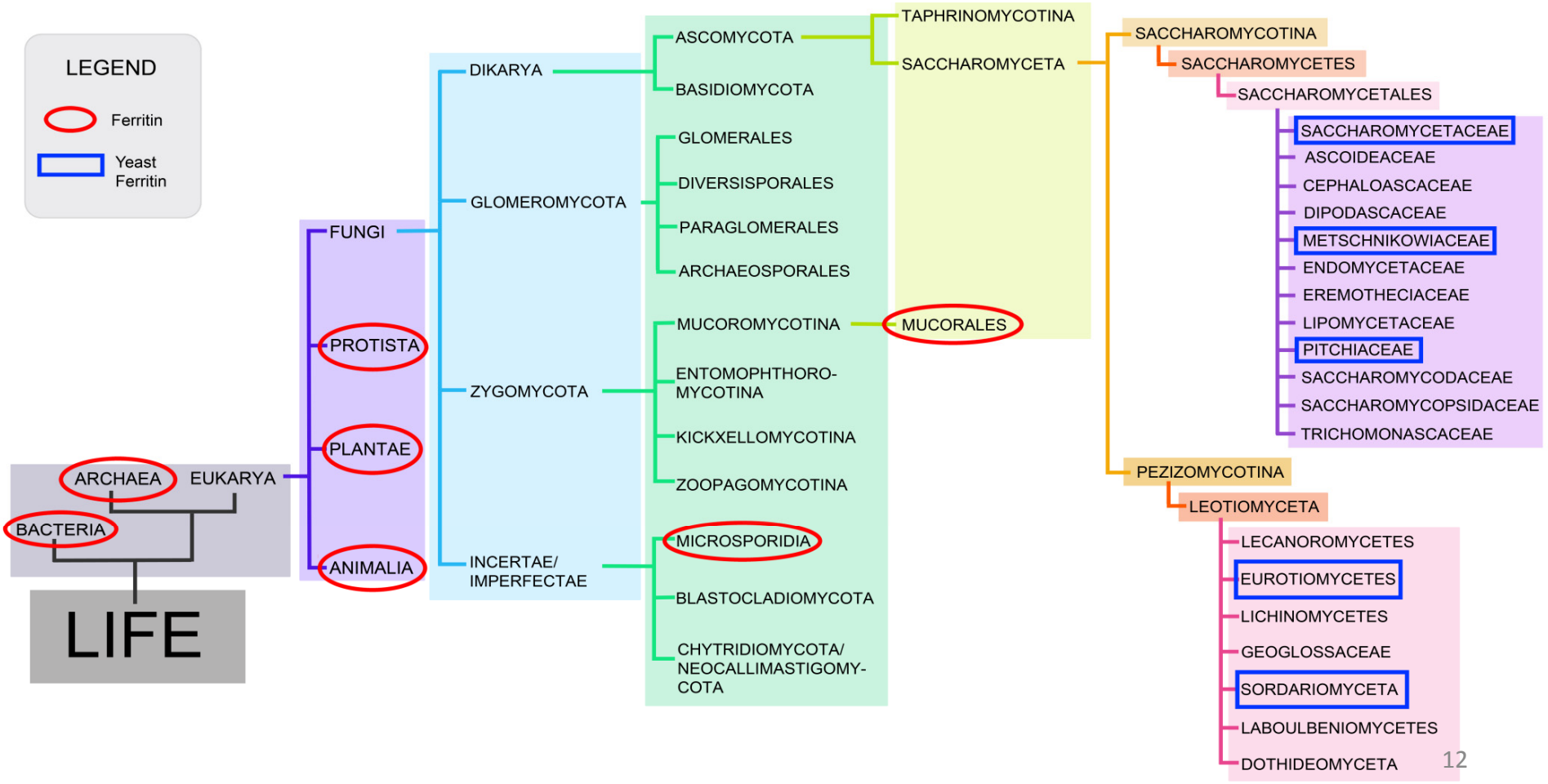
•One notable difference is the absence of the ferritin iron storage proteins in many fungi including yeast {Canessa, 2013}

•This suggest that human ferritn works on its own in yeast or that yeast has an unidentified ferritin like protein



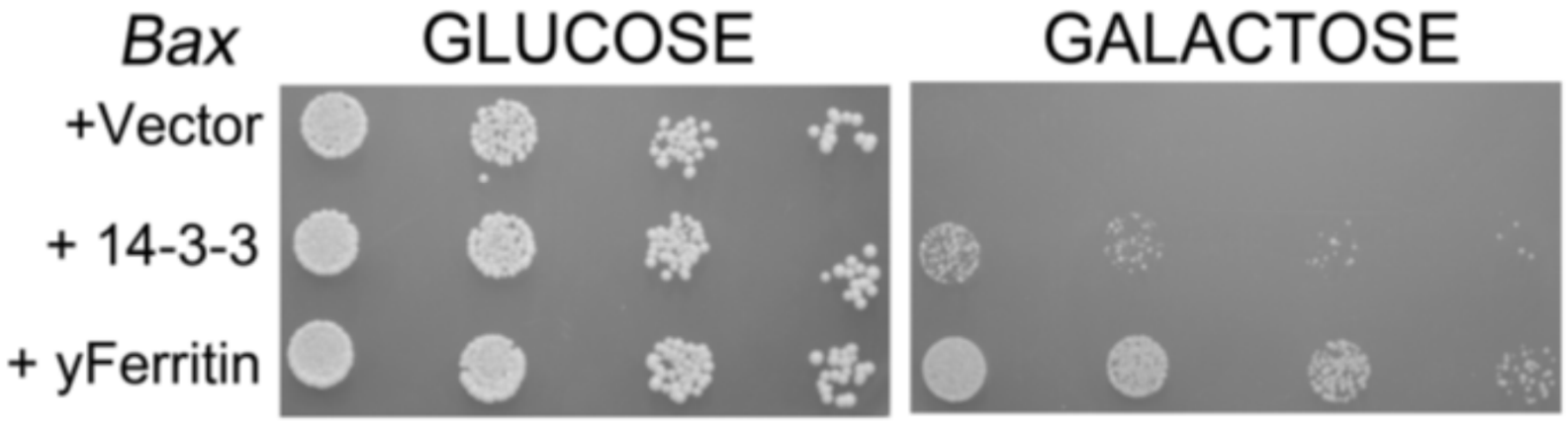
Adapted from BA et al. 2009 Metallomics **1**, 292-311; Aroun et al 2013Photochemical & Photobiological Sciences 11, 118-134; Saliba et al. 2015 J Blood Med. 6: 197–209.

Distribution of ferritins



Yeast may have a ferritin-like protein

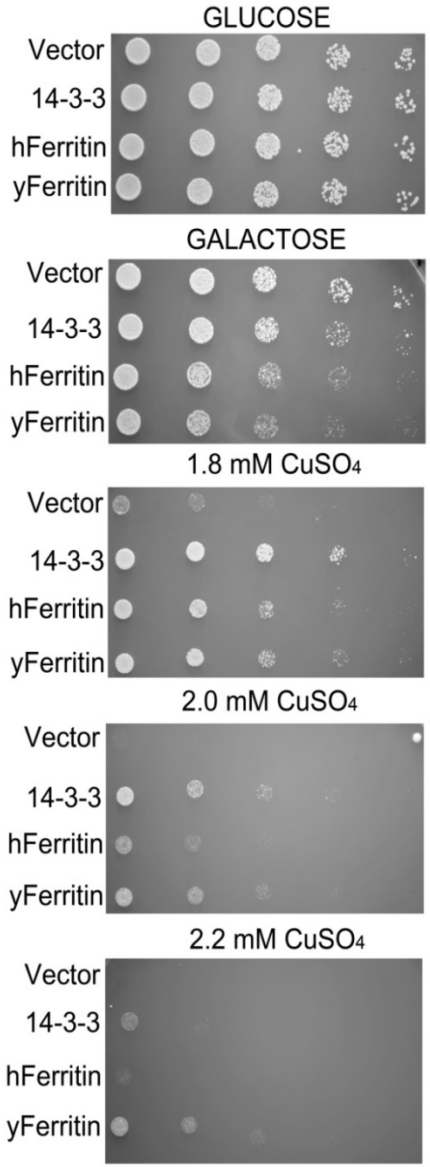
- Putative yeast ferritin shares 20% identity with hFerritin
- yFer is a protein of unknown function
- Like human ferritin, yfer is a Bax suppressor

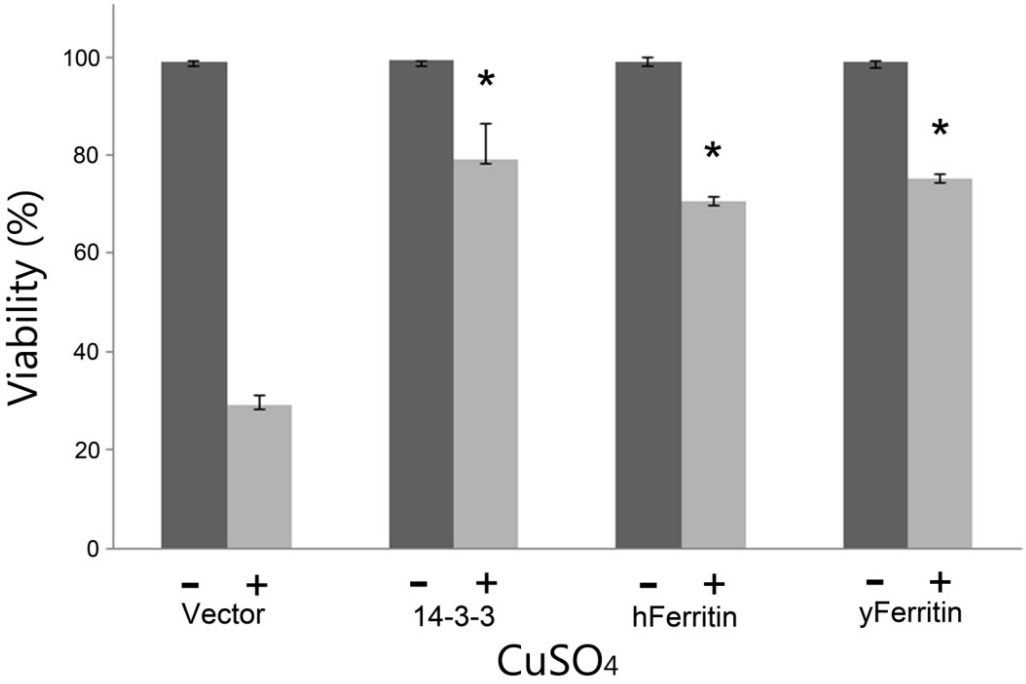


What are the functions of Ferritin in mammalian cells?

1. Human Ferritin Prevents ROS/PCD
2. Human Ferritin Stores Iron
3. High expression of ferritin induce iron starvation response
4. Altered gene doseage of ferritin will alter a the response to iron mediated PCD

1- Human and yeast Ferritin prevent copper mediated PCD

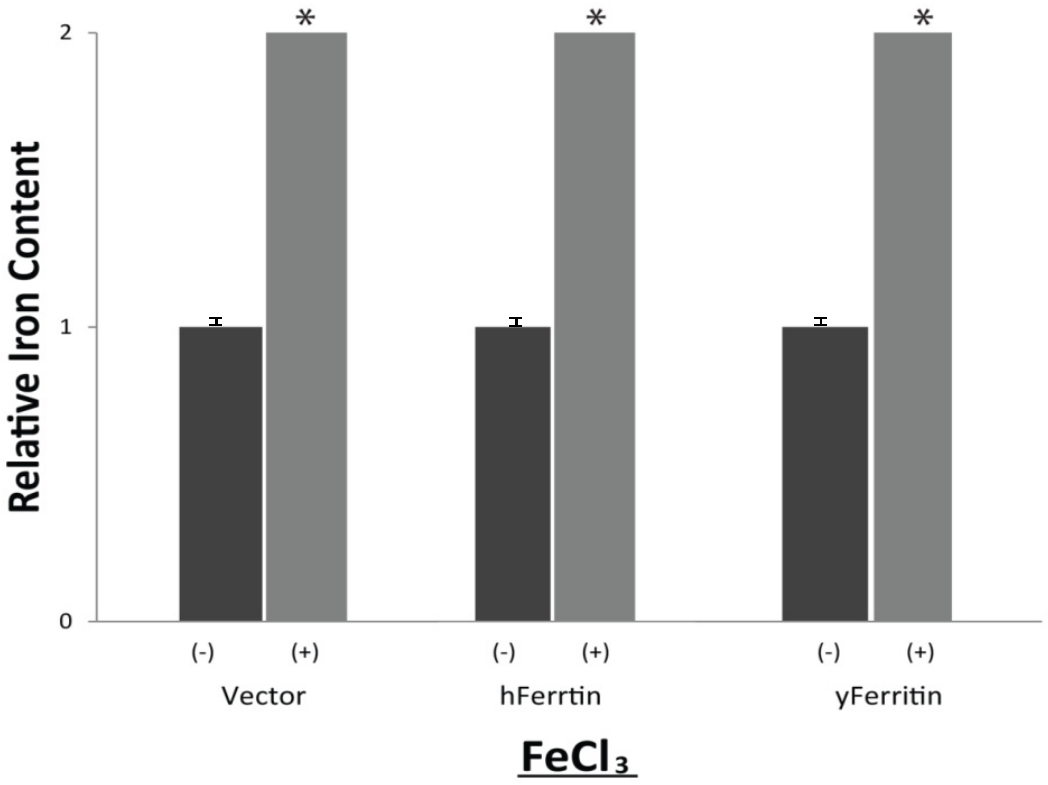




2- Ferritins do not increase iron storage in yeast

•Control cells as well as cells overexpressing ferritins grown in media with 10-fold increase in iron show a 2-fold in iron content.

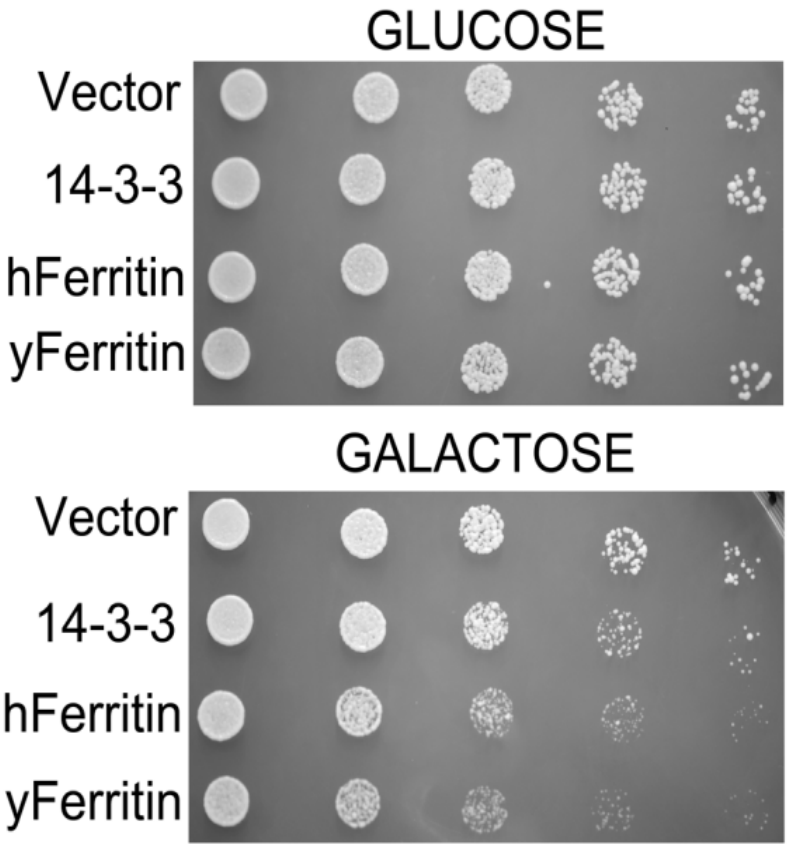
•No significant increase in relative iron content storage occurring due to the presence of ferritin.



3- Ferritins induce iron starvation response

In spot assays:

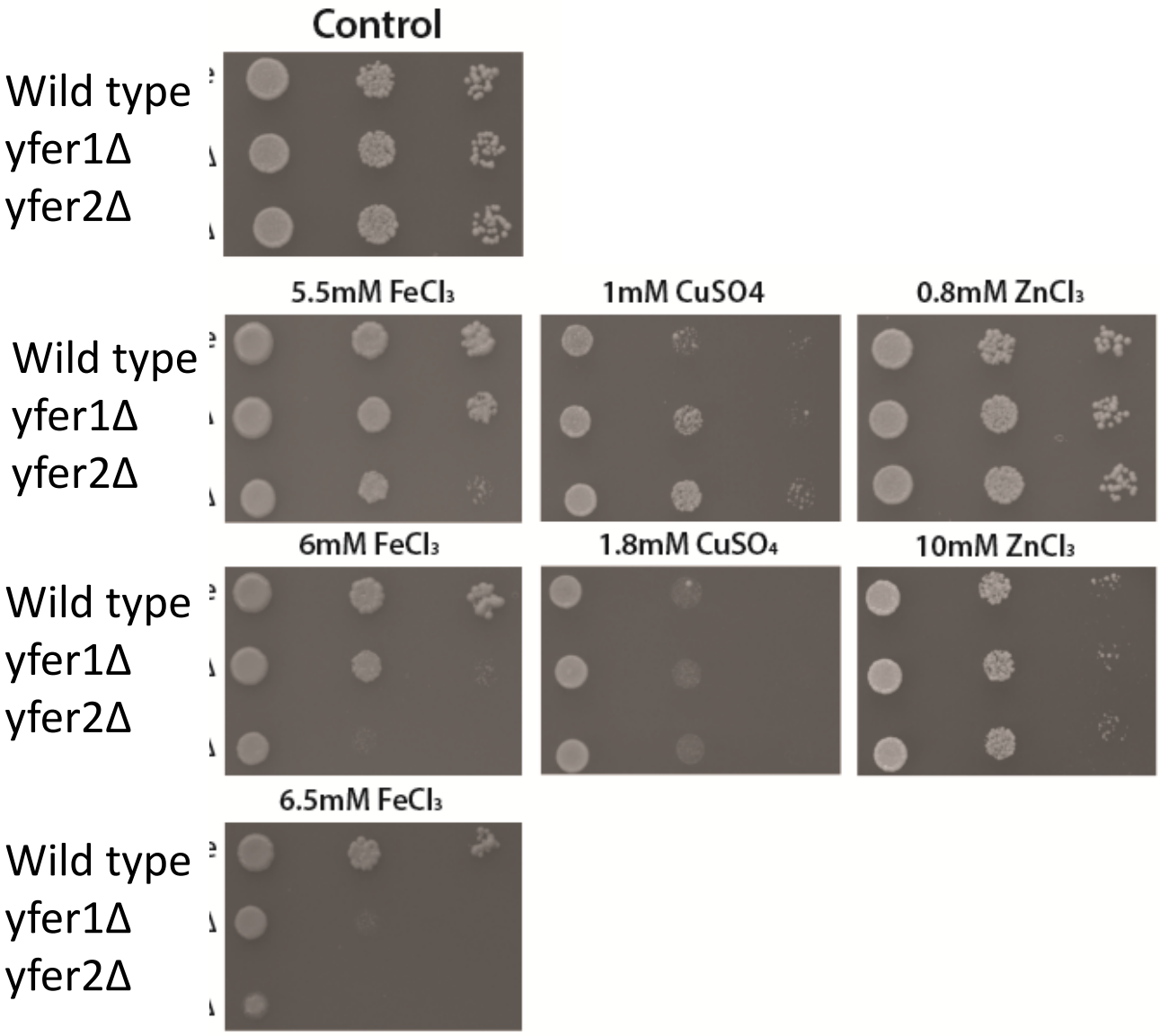
decrease in the growth on nutrient agar plates of yeast cells expressing ferritins.



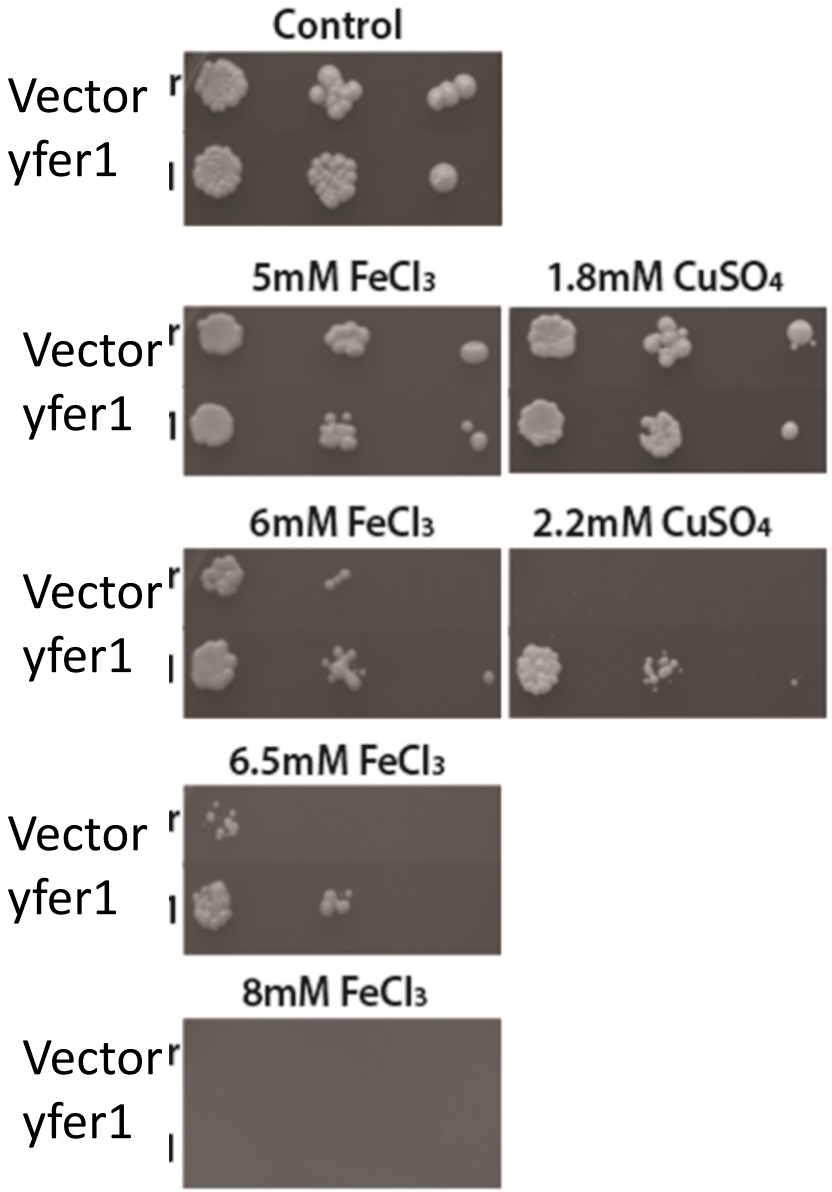
The doubling time:

control cells is 202 min ± 11 vs 277 min ± 10 in cells overexpressing human ferritin

4-Loss of yFer causes increased iron sensitivity

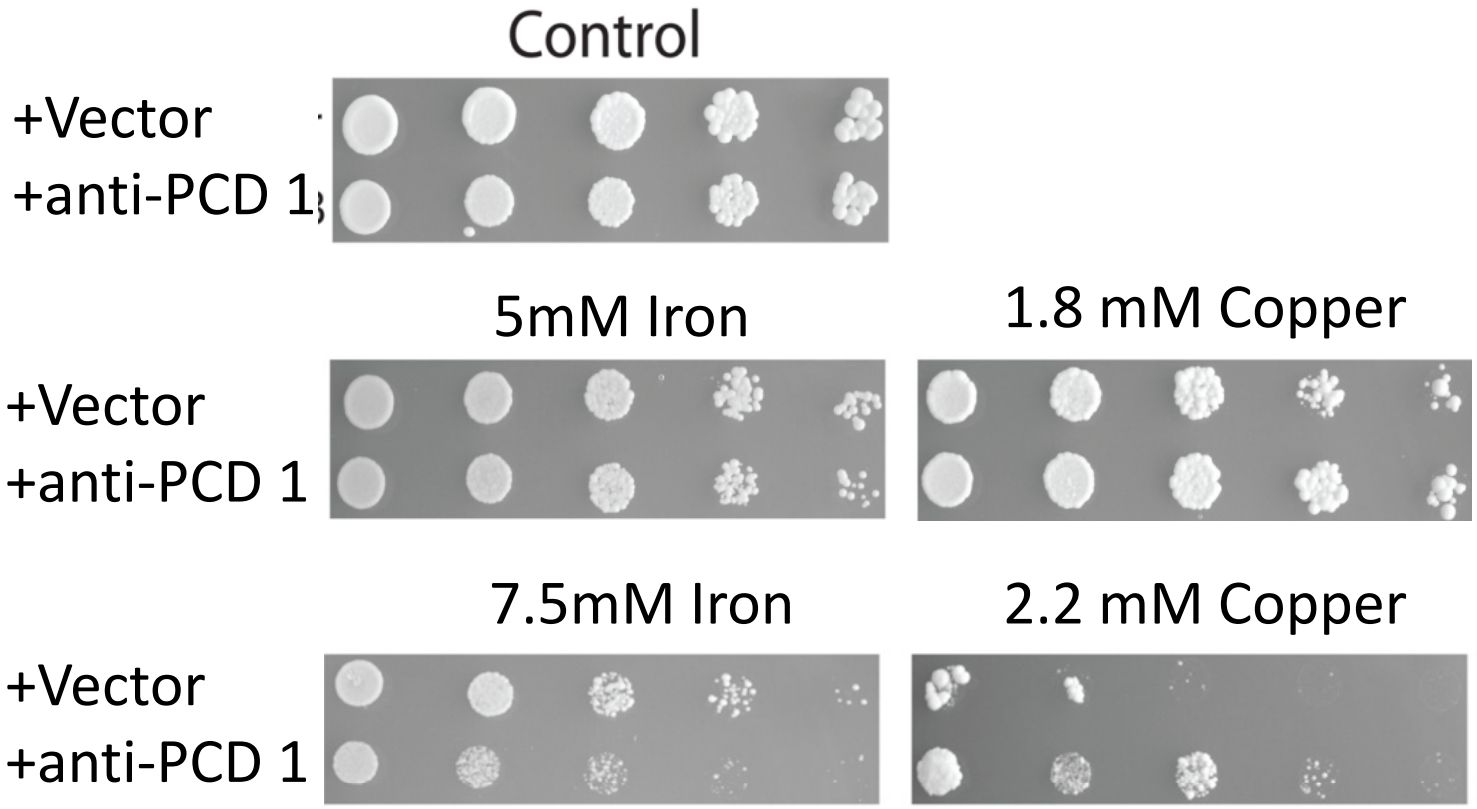


4-Overexpressing yFer increases iron resistance

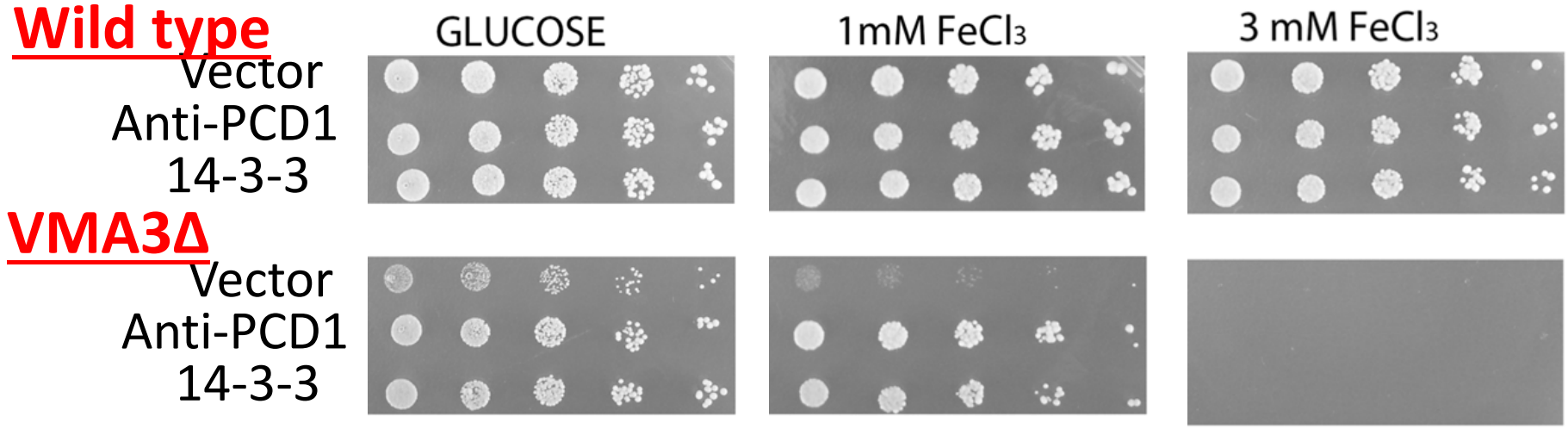


Surprisingly...

Blocking PCD specifically enhances iron mediated death



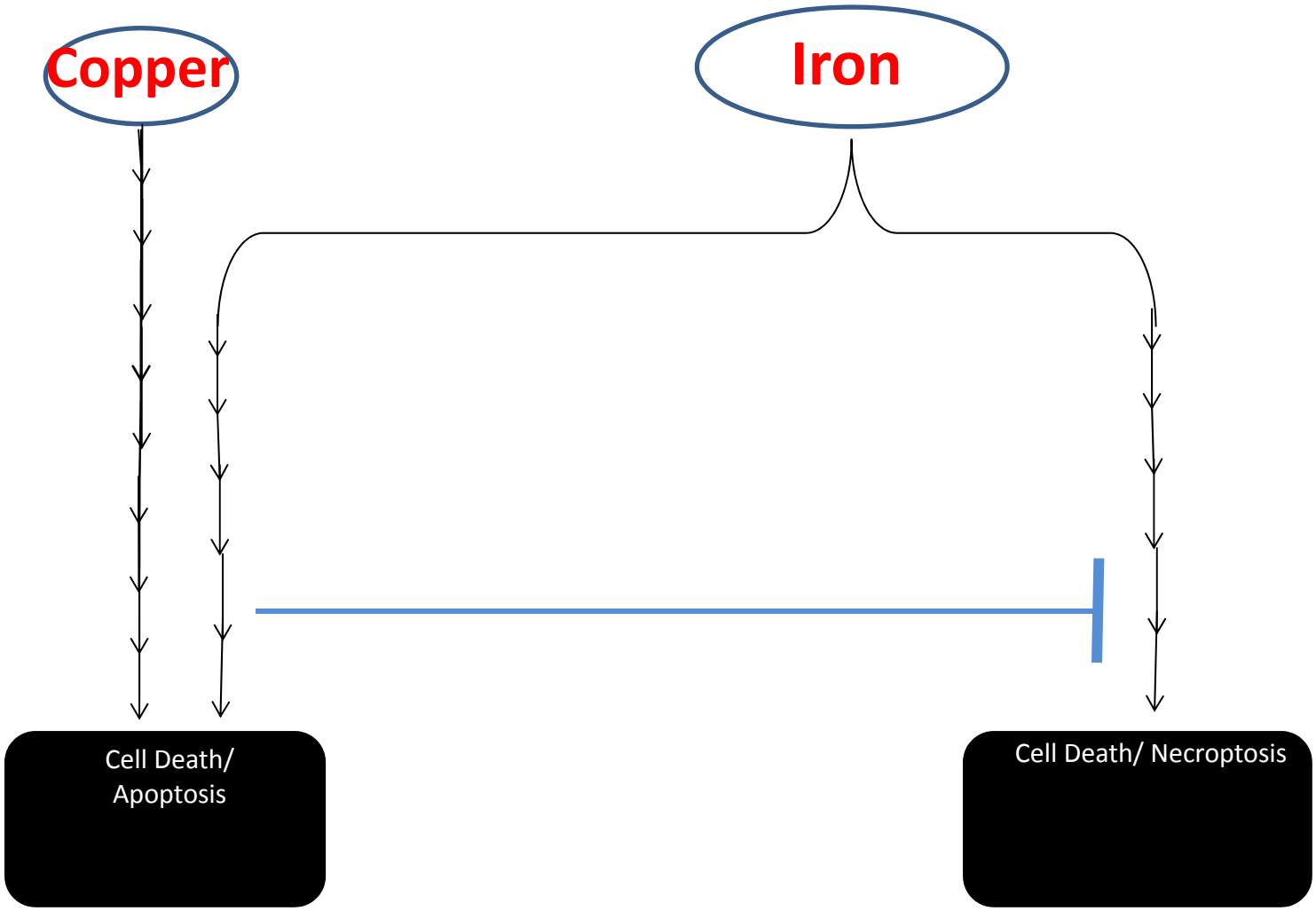
Anti-PCD and iron mediated cell death is vacuolar dependant



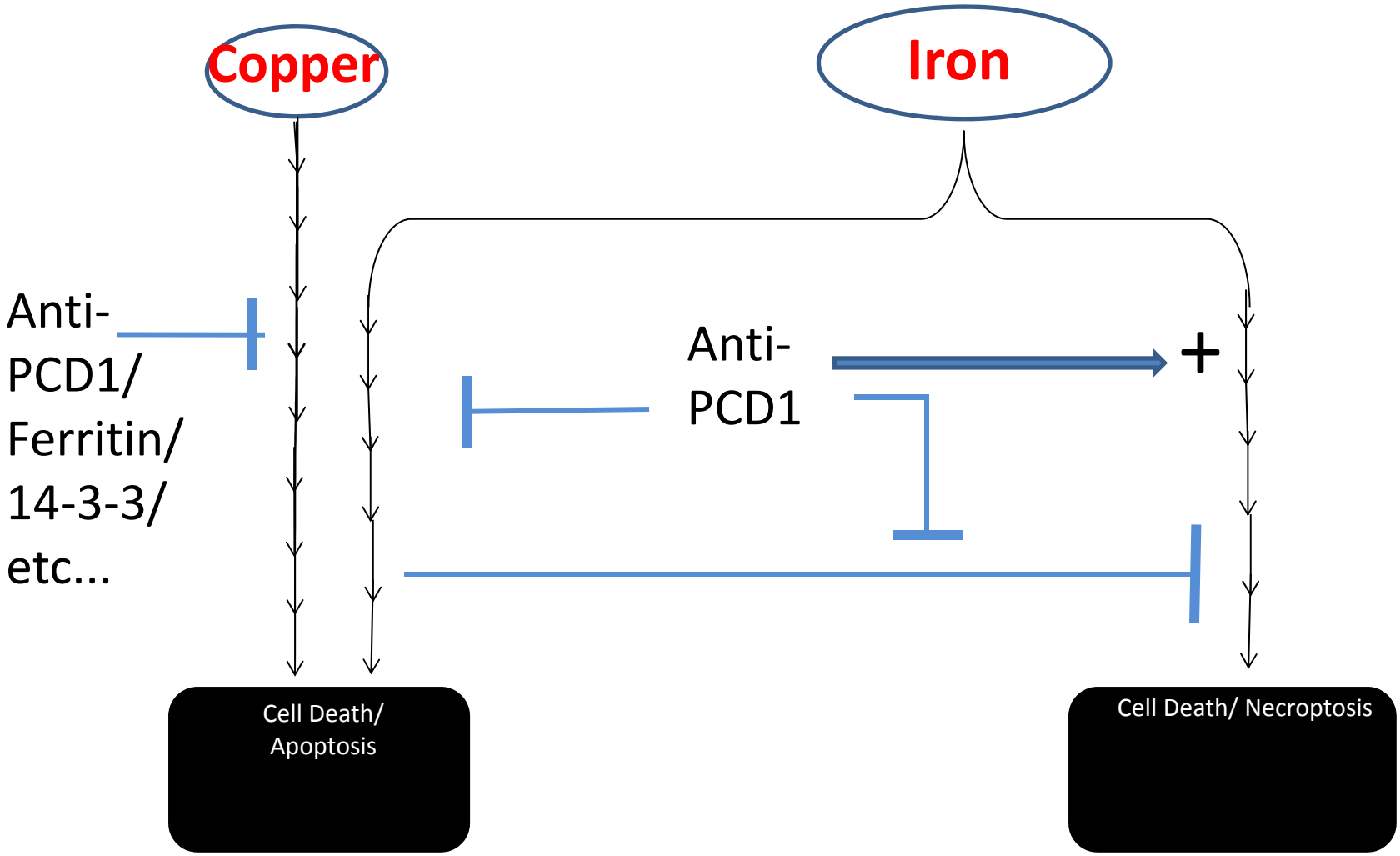
VMA3Δ mutants are supersensitive to iron but they are protected by anti-PCD1

This indicates that iron causes only one type of PCD in cells lacking a functional vacuole

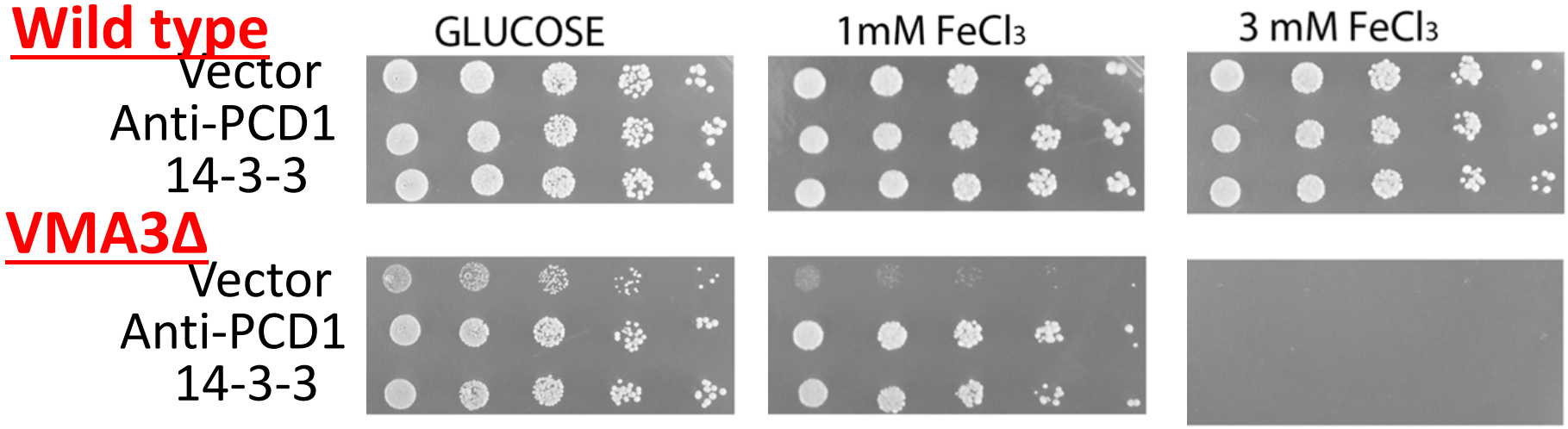
Hypothesis:
iron activates two PCD pathways



Blocking apoptosis enhances the necroptosis/alternative pathway



Anti-PCD1 and iron mediated cell death is vacuolar dependant



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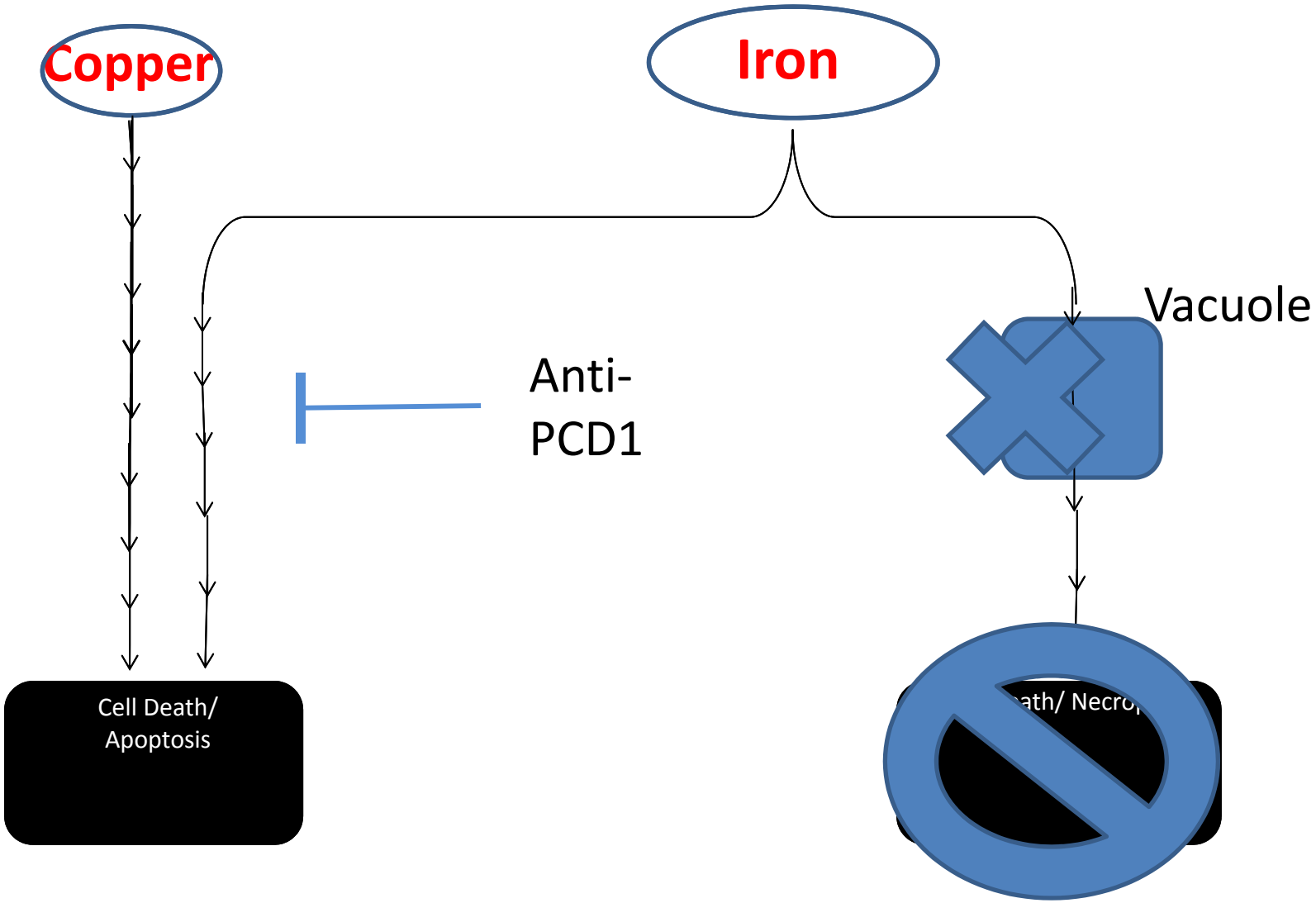
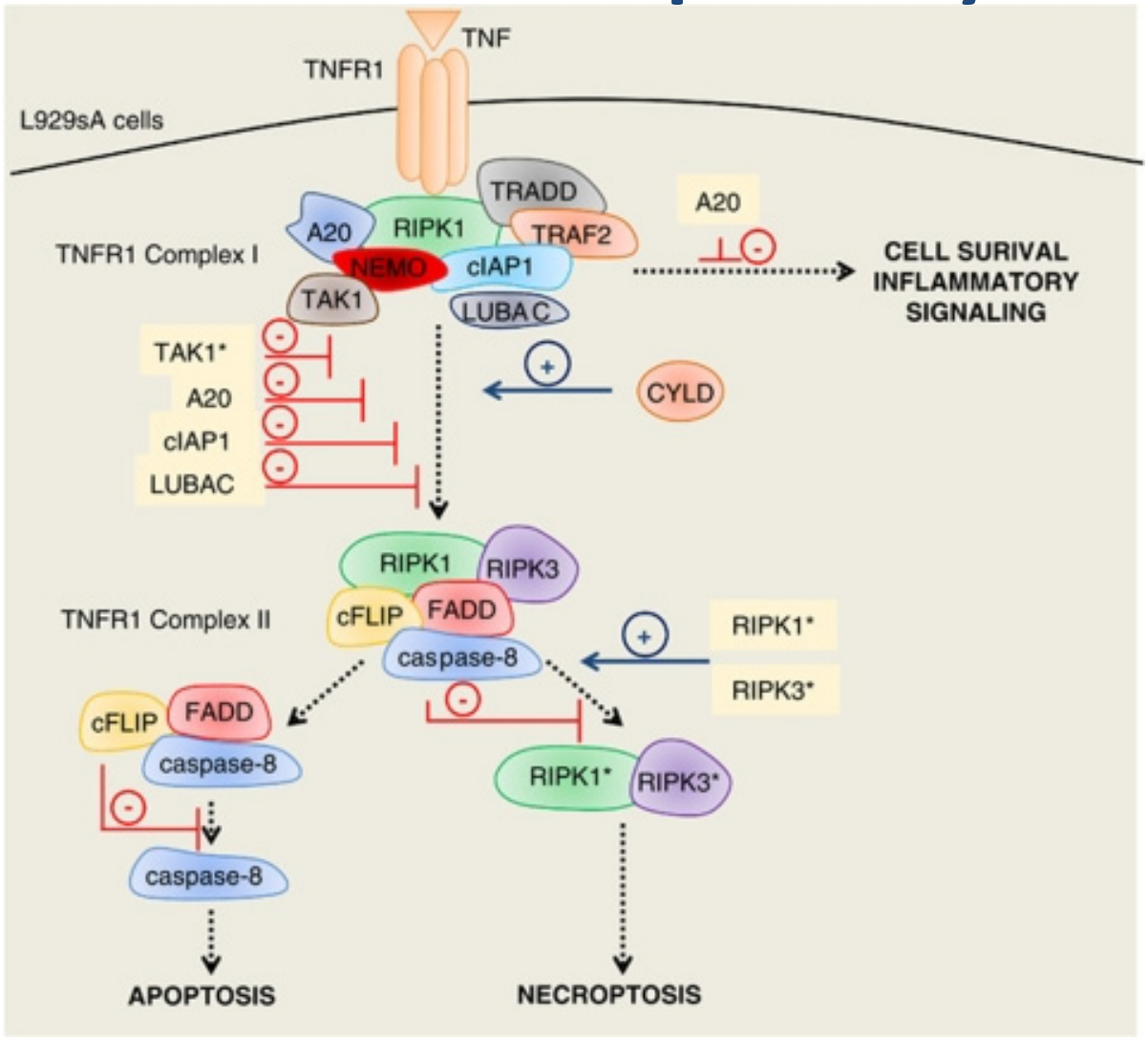
Loss of vacuole promotes iron mediated apoptosis



Blocking PCD can activate alternative death pathways



Vanlangenakker et al. 2012 Many stimuli pull the necrotic trigger, an overview. Cell Death Differ. 9:75-86.

"<u>Breaks and gears on TNF-induced necroptosis:</u>
The composition of TNFR1 complex II determines the cell death outcome: apoptosis or necroptosis.

Within TNFR1 complex II, the apoptotic machinery FADD, c-FLIP and caspase-8 suppresses the induction of necroptosis, which requires the kinase activity of RIPK1 and RIPK3."

CONCLUSIONS

- yFer may represent a yeast ferritin since it shares functional similarities to human ferritin and it is involved in iron metabolism
- Iron activates two distinct PCD inducing pathways in yeast
- Anti-PCD1 selectively inhibits one iron induced pathway
- The vacuole is critical for the other iron induced PCD pathway

Thank you!

THERE ARE THREE MAJOR PATHWAYS OF PCD

- **Type I or Apoptosis**

physiological response to specific suicide signals, or lack of survival signals

- **Type II or Autophagy**

Multifunctional process,

- Is essential for cellular maintenance, cell viability differentiation and development
- Is one of the mechanism of PCD that is accompanied by a massive cytoplasmic vacuolization

- **Type III or necrosis (necroptosis)**

- catastrophic form of death
- Chromatin clumps
- Mitochondria swell and rupture