

Expression of mammalian Onzin and Fungal Cadmium Resistance 1 in *S. cerevisiae* suggests ancestral functions of PLAC8 proteins in regulating mitochondrial metabolism and DNA damage repair

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Short Title: PLAC8 proteins regulate yeast mitochondrial functions and reduce DNA damage

21 Abstract

22 Protein domains are structurally and functionally distinct units responsible for particular protein
 23 functions or interactions. Although protein domains contribute to the overall protein function(s) and
 24 can be used for protein classification, about 20% of protein domains are currently annotated as
 25 “domains of an unknown function” (DUFs). DUF 614, a cysteine-rich domain better known as
 26 PLAC8 (Placenta-Specific Gene 8), occurs in proteins found in the majority of Eukaryotes. PLAC8-
 27 containing proteins play important yet diverse roles in different organisms, such as control of cell
 28 proliferation in animals and plants or heavy metal resistance in plants and fungi. For example,
 29 Onzin from *Mus musculus* is a key regulator of cell proliferation, whereas FCR1 from the
 30 ascomycete *Oidiodendron maius* confers cadmium resistance. Onzin and FCR1 are small, single-
 31 domain PLAC8 proteins and we hypothesized that, despite their apparently different role, a
 32 common molecular function of these proteins may be linked to the PLAC8 domain. To address this
 33 hypothesis, we compared these two PLAC8-containing proteins by heterologous expression in the
 34 PLAC8-free yeast *Saccharomyces cerevisiae*. When expressed in yeast, both Onzin and FCR1
 35 improved cadmium resistance, reduced cadmium-induced DNA mutagenesis, localized in the
 36 nucleus and induced similar transcriptional changes. Our results support the hypothesis of a
 37 common ancestral function of the PLAC8 domain that may link some mitochondrial biosynthetic
 38 pathways (i.e. leucine biosynthesis and Fe-S cluster biogenesis) with the control of DNA damage,
 39 thus opening new perspectives to understand the role of this protein domain in the cellular biology
 40 of Eukaryotes.

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42 Author Summary

43 Protein domains are the functional units of proteins and typically have distinct structure and
 44 function. However, many widely distributed protein domains are currently annotated as "domains of
 45 unknown function" (DUFs). We have focused on DUF 614, a protein domain found in many
 46 Eukaryotes and better known as PLAC8 (Placenta-Specific Gene 8). The functional role of DUF

614 is unclear because PLAC8 proteins seem to play important yet different roles in taxonomically distant organisms such as animals, plants and fungi. We used *S. cerevisiae* to test whether these apparently different functions, namely in cell proliferation and metal tolerance, respectively reported for the murine Onzin and the fungal FCR1, are mediated by the same molecular mechanisms. Our data demonstrate that the two PLAC8 proteins induced the same growth phenotype and transcriptional changes in *S. cerevisiae*. In particular, they both induced the biosynthesis of the amino acid leucine and of the iron-sulfur cluster, one of the most ancient protein cofactors. These similarities support the hypothesis of an ancestral function of the DUF 164 domain, whereas the transcriptomic data open new perspectives to understand the role of PLAC8-proteins in Eukaryotes.

57

58 Introduction

59 The PLAC8 domain was described for the first time in the protein Onzin, the product of the human
60 Placenta-Specific Gene 8 [1]. The same domain was later identified in many eukaryotes, but its
61 biological role remains elusive because PLAC8 proteins seem to play diverse roles in different
62 organisms and cell types. The mammalian Onzin has been reported as a repressed target of the c-
63 Myc oncoprotein [2], with pro-proliferative anti-apoptotic effects in many cell types and a role in
64 leukemia [3], hepatic, pancreatic [4, 5] and colon cancer progression [6, 7], but also in adipocyte
65 growth [8]. The same protein has pro-apoptotic activity in other cell types [9], indicating that the
66 final effect of Onzin is highly dependent on cell type. In plants, PLAC8 genes are also involved in
67 cell proliferation because mutants are altered in organs size. The tomato Fruit Weight 2.2 (FW2.2)
68 negatively influences fruit size and is downregulated in domesticated species [10]. Similar function
69 has been reported for FW2.2-like genes in other plant species, both dicots [11-14] and monocots
70 [15,16].

71 Another cellular role of PLAC8 proteins in plants and fungi is to increase resistance to heavy
72 metals. First described in *Arabidopsis thaliana*, the Plant Cadmium Resistance (PCR) protein
73 family includes proteins that confer resistance to cadmium or zinc. AtPCR1 was suggested to be -
74 or to be part of- a cadmium transporter because of its localization on the plasma membrane in both
75 *A. thaliana* and *S. cerevisiae* [17], while AtPCR2 was associated to zinc transport [18]. In fungi, the
76 only PLAC8 genes characterized to date are two Fungal Cadmium Resistance (FCR) genes
77 identified in a metal tolerant isolate of the mycorrhizal ascomycete *Oidiodendron maius* [19, 20],
78 which increased cadmium resistance when expressed in *S. cerevisiae*. In particular, OmFCR1
79 (hereafter FCR1) localized in the yeast nucleus and physically interacted with Mlh3p, a key player
80 in meiotic crossing-over and a subunit of the DNA mismatch repair (MMR) complex [19].

81 Despite the numerous studies on PLAC8 proteins in different organisms, it is still unclear whether
82 the PLAC8 domain has a molecular function common to all biological systems. To address this
83 question, we have used *S. cerevisiae* as a model organism to investigate two small, single-domain

PLAC8 proteins from taxonomically distant organisms, the mammalian Onzin from *Mus musculus* and the fungal FCR1 from *O. maius*. *S. cerevisiae* is a good model system because it has been successfully used to test plant and fungal PLAC8 gene functions by heterologous expression [17-20]. In addition, although *S. cerevisiae* lacks genes coding for PLAC8 proteins this protein domain can be found in the genome of some basal fungi and other members of Saccharomycotina (see Mycosm, <https://genome.jgi.doe.gov/programs/fungi/index.jsf>), thus suggesting that the metabolic framework interacting with this protein domain is found early in fungal evolution. *S. cerevisiae* genome lacks PLAC8 genes, but it

When expressed in cadmium-exposed *S. cerevisiae*, Onzin and FCR1 displayed very similar phenotypes, as they both conferred cadmium resistance and increased cell proliferation and vitality. Both proteins localized in the yeast nucleus, reduced the mutation frequency in homonucleotide runs and determined similar transcriptomic changes in cells exposed to cadmium. In particular, both PLAC8 proteins up-regulated ancient and conserved metabolic pathways that link mitochondrial functions related to leucine biosynthesis, Fe-S cluster biogenesis and maintenance of nuclear DNA integrity. Although the exact role of the PLAC8 domain remains unclear, our findings provide support to the hypothesis of a common ancestral function for this protein domain.

Results and discussion

Onzin is a small protein highly conserved in all vertebrates and in particular in mammals, where mouse and human orthologous proteins are 83% identical (Fig 1). By contrast, Onzin shared only 29 amino acids with the fungal PLAC8 protein FCR1, with an overall 25% sequence identity mainly confined to the signature cysteine-rich motif of the PLAC8 domain (Fig 1).

A first set of experiments was aimed to verify whether Onzin expression in cadmium-exposed *S. cerevisiae* induced a phenotype similar to the one previously described for FCR1 [19]. Spot dilution assays at two CdSO₄ concentrations (Fig 2A) showed that both Onzin and FCR1 confer cadmium tolerance to *S. cerevisiae*, when compared with the control strain transformed with the empty

vector. At 25 μ M CdSO₄, Onzin-expressing yeast grew even better than the FCR1-expressing strain (Fig 2A). A truncated Onzin lacking the conserved N-terminal region of the PLAC8 domain (Onzin ^{Δ 28-38}) led to a partial loss-of-function phenotype (Fig 2A), suggesting that this protein region is important for Onzin function. Evaluation of the cadmium half inhibitory concentration (IC₅₀) on the same yeast strains grown in liquid culture yielded fully consistent results (S1 Fig). Yeast growth and viability was also monitored for 24 h in liquid culture on control medium and on medium containing 25 μ M CdSO₄. On control medium, all yeasts strains showed identical growth curves, as measured by OD₆₀₀, whereas on cadmium-amended medium Onzin and FCR1 expressing yeasts grew more than the control strain, starting from 6 h incubation (Fig 2B). Cell survival in the same growth experiment was measured by colony forming units (CFUs) count and was similar for all yeast strains grown on control medium. By contrast, Onzin and FCR1 expression led to CFU numbers higher than the empty vector after 6 and 8 h of cadmium exposure (Fig 2C). At later time points, cell survival on cadmium-amended medium was low for all yeast strains (Fig 2C). Subcellular Onzin localization (Fig 3) in yeast cells by C-terminal tagging with the Enhanced Green Fluorescent Protein (EGFP) showed that Onzin-EGFP co-localized, like FCR1-EGFP, with the Red Fluorescent Protein (RFP) fused to the nuclear localization signal (NLS). Thus, this first set of experiments showed, for Onzin-expressing yeast, the same phenotype already described for FCR1 [19]. FCR1 and Onzin expression increased yeast growth exclusively on cadmium-containing medium, indicating a protein function only measurable during cadmium stress. The nuclear localization of EGFP-tagged FCR1 and Onzin would exclude a direct role in metal detoxification mechanisms, such as membrane transport or scavenging. We therefore investigated possible functions of the two PLAC8 proteins correlated with their nuclear localization.

134 **Both PLAC8 proteins physically interact with Mlh3p and reduce cadmium induced DNA** 135 **mutations**

136 Cadmium does not damage DNA directly but is mutagenic because it interferes with the cellular
137 response to DNA damage [21] and inhibits all major DNA repair pathways, including the mismatch
138 repair (MMR) complex [21-23]. FCR1 was found to physically interact with Mlh3p, a component
139 of the MMR complex [19] and a yeast two hybrid assay confirmed its interaction with the C-
140 terminal region of both the yeast and the *O. maius* Mlh3p (S2 Fig). The same assay revealed a
141 similar, albeit weaker, interaction of Onzin with the C-terminal region of the yeast and the mouse
142 Mlh3 proteins (S2 Fig).

143 Previous experiments using the forward mutation assay at the canavanine-resistance (*CAN1*) locus
144 did not reveal an influence of FCR1 on the DNA mutation rate under cadmium stress [19].
145 However, the *CAN1* assay could reveal only small differences between wild-type and a *mlh3*-
146 defective yeast [24]. Here, we investigated the influence of FCR1 and Onzin expression on
147 cadmium-induced mutagenicity with the more sensitive yeast *lys2::insE-A₁₄* reversion assay, based
148 on the restoration of the open reading frame in a mononucleotide run of 10 adenines within the
149 *lys2::insE-A₁₄* allele [25]. In cells defective of the MMR system, this assay could reveal 10- to
150 1,000-folds increase in mutations [26]. On control medium, yeast strains transformed with the
151 empty vector or with the two PLAC8 genes showed no differences in DNA mutation rate (Table 1).
152 As expected, cadmium exposure increased the DNA mutation rate, but expression of both FCR1
153 and Onzin reduced cadmium-induced DNA mutagenesis about three-folds when compared with the
154 empty vector (Table 1). As FCR1 and Onzin physically interact with Mlh3p, a *mlh3* defective
155 mutant was used to investigate whether reduction in the mutation rate required this protein. The
156 *mlh3* strain transformed with the empty vector showed a higher background mutation rate both in
157 control medium and in cadmium-amended medium, as already shown [23], but expression of FCR1
158 and Onzin led to a five-fold reduction in the DNA mutation rate when this mutant was exposed to
159 cadmium (Table 1). Thus, yeast cells exposed to cadmium display a reduced DNA mutation rate

when they express either FCR1 or Onzin, although the relationship between these two PLAC8 proteins and the MMR complex remains unclear.

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Table 1. Influence of Onzin and FCR1 on DNA mutation rate measured by the Lys2::insE-A₁₄ reversion assay.

Strain	Vector	Treatment	Reversion Rate x 10 ⁻⁵ (confidence intervals)	Fold increase in mutation rate ^a	n ^b
WT	pFL61-EV	Control medium	0,14 (0,30÷0,09)	1	30
	pFL61-FCR1		0,19 (0,21÷0,13)	0,74	20
	pFL61-Onzin		0,18 (0,20÷0,16)	0,74	20
WT	pFL61-EV	CdSO ₄ 1μM	5,49 (6,10÷5,00)	1	30
	pFL61-FCR1		1,75 (1,88÷1,54)	3,14	20
	pFL61-Onzin		1,92 (1,99÷1,70)	2,85	20
mlh3	pFL61-EV	Control medium	1,55 (1,88÷1,03)	1	25
	pFL61-FCR1		1,63 (1,80÷1,20)	0,95	25
	pFL61-Onzin		1,57 (1,68÷1,04)	0,99	25
mlh3	pFL61-EV	CdSO ₄ 1μM	14,61 (15,11÷14,01)	1	25
	pFL61-FCR1		2,8 (3,01÷2,03)	5,21	25
	pFL61-Onzin		2,55 (2,97÷2,12)	5,72	25

Reversion to Lys⁺ phenotype was assessed in the wild-type (WT) and *mlh3* yeast mutant strains expressing FCR1, Onzin or the empty vector (EV) on both control medium and cadmium-containing medium. ‘n’ column indicates the number of independent cultures tested from at least two independently constructed strains. Median mutation rates are presented as x10⁻⁵ with 95%

confidence intervals. Relative mutation rates are compared with the empty vector of the same genetic background strain.

^aeach median reversion rate was normalized to the empty vector median rate to calculate the fold increase; ^bn=number of independent replicates.

173

174 **Onzin and FCR1 induce similar transcriptomic changes in cadmium-exposed yeast**

175 To identify the yeast pathways transcriptionally regulated by the two PLAC8 proteins, an RNAseq
176 experiment was performed after 8 h of exposure to 25 μ M CdSO₄. The diagram in S3 Fig reports
177 the number of transcripts significantly regulated in yeast expressing either FCR1 or Onzin, as
178 compared with the empty vector (log₂ fold-change threshold >1 or <-1, adjusted p-value <0.05).
179 Genes up-regulated by both proteins, indicated as *PLAC8 up-regulated* genes, represented 68% and
180 70% of the total number of genes up-regulated by FCR1 and Onzin, respectively. Genes down-
181 regulated by both proteins, indicated as *PLAC8 down-regulated* genes, represented 59% and 72% of
182 the total number of genes down-regulated by FCR1 and Onzin, respectively. A complete list of
183 FCR1 and Onzin regulated genes is available in the S1 Table.

184 “Mitochondrion” and “mitochondrial parts” were the only Gene Ontology (GO) enriched cellular
185 compartments identified among *PLAC8 up-regulated* genes, together with the biological processes
186 involved in the biosynthesis of the branched-chain amino acids, and the metal ion binding
187 molecular functions, in particular iron (S2 Table). Consistently with these GO data, *PLAC8-*
188 *upregulated* genes were involved in several mitochondrial pathways. “Plasma membrane” was the
189 cellular compartment enriched in *PLAC8 down-regulated* genes , together with ions, amino acids
190 and sugars transport molecular functions, and iron homeostasis (S2 Table).

191

192 **Onzin and FCR1 do not activate antioxidant responses to cadmium**

193 Activation of antioxidative enzymes and metabolites is a common cell defense response that could
194 reduce cadmium toxicity [27]. Although cadmium is unable to generate free radicals directly,

cadmium exposure induces in fact the production of reactive oxygen species (ROS). Activation of antioxidative enzymes and metabolites is therefore a common cell defense response that could reduce cadmium toxicity [27]. Previous experiments [19] suggested that FCR1 does not confer cadmium tolerance by increasing the antioxidative cell potential, and our transcriptomic data confirm this observation, as no genes coding for enzymes or compounds involved in ROS scavenging (e.g. superoxide dismutases or enzymes involved in glutathione metabolism) were identified among the *PLAC8 up-regulated* genes (S1 Table).

The most *PLAC8 up-regulated* gene was *ALD5*, coding for a mitochondrial K⁺ activated aldehyde dehydrogenases (ALDH). In yeast, ALDHs have a distinct role in the antioxidant cell responses because they maintain redox balance by supplying reducing equivalents in the form of NADH and NADPH [28]. However, Ald5p seems to play only a minor role as ALDH, because an *ald5* mutant retained 80% of K⁺-activated ALDH activity [29]. ALD4, the major K⁺-activated mitochondrial ALDH, as well as the cytosolic ALD6, were both *PLAC8 down-regulated* genes (S1 Table). Kurita & Nishida [29] showed a more important role of the mitochondrial Ald5p in the regulation of the biosynthesis of electron transport chain components. We measured total cellular respiration in the yeast strain W303-1B transformed with Onzin, FCR1 or the empty vector, and the results (S4 Fig) indicate that the overall oxygen consumption was slightly increased (about 10%) in the *PLAC8*-expressing strains, irrespective of CdSO₄ exposure. Overall, the transcriptomic data suggest that the two *PLAC8* proteins did not reduce cadmium toxicity and mutagenicity simply by increasing the cell antioxidative response.

215

Onzin and FCR1 induce iron-dependent pathways for leucine and iron-sulfur cluster biosynthesis

The *PLAC8 up-regulated* genes included key genes involved in amino acid biosynthesis, such as arginine, histidine, methionine and threonine (S1 Table), but one of the most represented pathways was the super-pathway of leucine, isoleucine, and valine biosynthesis (S2 table, Fig 4). *PLAC8 up-*

221 *regulated* genes included *ILV2*, *ILV3*, *ILV5* and *BAT1*, involved in common reactions of branched-
222 chain amino acids (BCAAs) biosynthesis, and genes specific for the leucine pathway (*LEU1*, *LEU2*,
223 *LEU4*, *LEU9* and *OAC1*). Oac1p, a mitochondrial oxaloacetate transporter, catalyzes the export to
224 the cytoplasm of α -isopropylmalate, an intermediate of leucine biosynthesis produced inside the
225 mitochondrion [30]. Together with *GDH1*, encoding a major enzyme for ammonia assimilation in *S.*
226 *cerevisiae*, all these *PLAC8 up-regulated* genes are established or potential members of the Leu3p
227 regulon [31], which is transcriptionally regulated by Leu3p. Two *PLAC8 up-regulated* genes of the
228 Leu3p regulon, the acetohydroxy acid reductoisomerase *ILV5* and the BCAA aminotransferase
229 *BAT1* have an additional transcriptional control by Tpk1p, a subunit of yeast protein kinase A,
230 thought to have a role in controlling mitochondrial iron homeostasis [32].

231 The mitochondrion plays a focal role in iron metabolism because is a major generator of heme and
232 iron-sulfur clusters (ISC) cofactors [33]. ISC are among the most ancient and versatile cofactors of
233 proteins involved in many cellular processes such as respiration, DNA synthesis and repair,
234 metabolite biosynthesis, and oxygen transport catalysis [34-37]. Biogenesis of the ISC in
235 Eukaryotes is a highly conserved process that involves the mitochondrial ISC assembly machinery,
236 an export system from the mitochondrion and the cytosolic ISC assembly (CIA) machinery [36].

237 Notably, *PLAC8 up-regulated* genes included *ISU2* [38] and *DRE2* [39], coding for essential
238 proteins in the mitochondrial and the cytosolic ISC assembly machineries, respectively (S1 Table,
239 Fig 4).

240 Some *PLAC8 up-regulated* components of the BCAA biosynthetic pathway can influence ISC
241 cofactors biosynthesis in yeast. For example, mitochondrial ISC biosynthesis is regulated through
242 Leu1p, an abundant cytoplasmic ISC-containing enzyme [40] and a key regulator in the
243 mitochondrial-cytoplasmic ISC balance [41]. Leu1p shares high homology to the iron regulatory
244 protein Irf1 in mammalian cells, suggesting an influence on iron metabolism within the cell [42].

245 Iron deficiency is thought to influence the pathway of leucine biosynthesis by reducing the activities
246 of multiple ISC-containing enzymes, including Leu1p and Ilv3p [43].

Overall, the transcriptomic data clearly indicate, in cadmium-exposed yeast expressing both PLAC8 proteins, the up-regulation of leucine and ISC biosynthesis, two iron-dependent pathways that involve the mitochondrion.

Expression of both PLAC8 proteins in yeast does not modify intracellular iron content

A correlation between iron homeostasis and cadmium response has been revealed by genome-wide screening of *S. cerevisiae* deletion mutant collections, as many cadmium-sensitive mutants were affected in genes related to iron homeostasis [44, 45]. Cadmium interferes with iron homeostasis by reducing iron uptake, since iron addition rescued cadmium-sensitivity of yeast mutants [44] and increased cadmium tolerance of *S. cerevisiae* [46]. Moreover, cadmium exposure stimulated the expression of several yeast genes related to iron uptake [47].

The “iron regulon” is a group of ~30 genes mostly involved in iron acquisition, activated upon iron deficiency by the iron-sensing transcription factors Aft1p and Aft2p [43]. Several *PLAC8* down-regulated genes (S1 Table) are known members of the yeast iron regulon, like the high affinity iron uptake system (*FET3* and *FTR1*), components of the siderophore transport system (*FIT2* and *FIT3*, *SIT1*, alias *ARN3*), and the mRNA-binding protein *TIS11* (alias *CTH2*). Their expression pattern indicates an activation of the iron regulon in cadmium-exposed yeast cells expressing the empty vector, thus suggesting that they experience a condition of iron depletion, as compared with *PLAC8*-expressing yeasts. We therefore measured total iron content in yeast exposed to cadmium and expressing FCR1, Onzin or the empty vector. No statistical differences were found in the total iron content of yeast cells exposed for 8 h to 25 μ M CdSO₄, the same conditions used for the transcriptomic experiment, independently on the intracellular Cd-concentration (S3 Table). Although it is still possible that different cell compartments may experience different iron concentrations, these results indicate that the expression pattern of the iron regulon genes in the *PLAC8*-expressing yeast does not simply reflect an increased iron content. Chen and colleagues [48] demonstrated that inhibition of ISC biosynthesis induced the iron regulon in spite of high

cytosolic iron levels. Ueta and colleagues [49] later showed that an ISC-dependent interaction between monothiol glutaredoxins and Aft1p in *S. cerevisiae* specifically promotes the dissociation of Aft1p from its target promoters under iron sufficiency. Thus, we may speculate that the up-regulated expression of ISC biosynthesis in the two PLAC8-expressing yeast strains may similarly downregulate transcription of the iron regulon.

Phenotype of PLAC8-expressing yeast: suggestions from the transcriptome

Although the phenotype of Onzin and FCR1-expressing yeast exposed to cadmium included increased growth (Fig 2) and reduced DNA mutation rate (Table 1), no genes specifically related to cell cycle control or DNA damage repair could be identified among the *PLAC8-regulated* transcripts. Of course, we cannot exclude a post-transcriptional regulation of these pathways, but we could also speculate on other possible scenarios based on the transcriptomic data.

The main pathway up-regulated by both PLAC8 proteins in cadmium-exposed yeast was the leucine biosynthetic pathway. This pathway generates leucine, one of the most conserved and potent *TORC1* (target of rapamycin complex 1) activating growth signals (Fig 4). TORC1 is a protein complex, conserved throughout Eukaryotes, that functions as a master regulator of cell proliferation, survival and growth [50]. The mitochondrial branched amino acid transferase Bat1p and the β -isopropylmalate dehydrogenase Leu2p, both *PLAC8 up-regulated* genes, can reversibly metabolize respectively leucine or β -isopropylmalate to α -ketoisocaproate (KIC), another effective *TORC1* activator [51]. A mitochondrial GTP/GDP transporter (GGC1) up-regulated by both PLAC8 proteins has been also characterized as a component of the rapamycin/target of rapamycin (TOR) signaling pathway [52]. Lesuisse and colleagues [53] demonstrated that GGC1 mutants accumulated iron in the mitochondria and suggested a role in intracellular iron balance, either by directly transporting iron from mitochondria to cytoplasm as heme, Fe-S or other forms, or by transporting molecules that influence iron uptake and distribution. In yeast, TORC1 regulates cellular responses to a variety of environmental stresses and is a determinant of cell survival in

299 response to DNA damage thanks to increased dNTPs synthesis by ribonucleotide reductases (RNR,
300 [54]).

301 Several proteins involved in DNA synthesis and repair contain ISC cofactors, including replicative
302 DNA polymerases and primase, DNA helicases, nucleases, glycoylases and demethylases [55, 56].

303 An interesting mechanism proposed by Arnold and colleagues [57], named *DNA charge transport*,
304 suggests that DNA processing enzymes containing the ISC cofactor may use electrons released
305 from redox active iron to rapidly and efficiently scan DNA over long molecular distances for
306 mismatches and damages. It is therefore not surprising that impairments in the mitochondrial or the
307 cytosolic ISC assembly machineries are connected with nuclear genomic instability [58, 59].

308 Therefore, we could speculate that, by up-regulating ISC biosynthesis, yeast cells expressing the
309 two PLAC8 proteins may increase their survival because they provide essential cofactors to
310 enzymes involved in DNA damage repair, thus reducing the high DNA mutation rate observed in
311 cadmium-exposed yeast cells (Table 1). MM19, a late-acting CIA component likely implicated in
312 the delivery of ISC into nuclear and cytosolic apoproteins [56], was found to be necessary to
313 *Schizosaccharomyces pombe* to grow on cadmium [60].

314 Although these hypotheses are only based on transcriptomic data, some experimental evidence
315 suggests a link between PLAC8 protein functions in cadmium tolerance, ISC biosynthesis and DNA
316 damage repair. The nuclear DNA damage caused by defects of ISC biosynthesis activates at least
317 two different signalling pathways that converge at Dun1p, a protein kinase that controls the DNA
318 damage response in yeast [61]. The DNA damage checkpoint mediated by the Mec1p–Chk1p–
319 Dun1p signaling transduction pathway was found to be activated by dysfunctions in ISC-targeting
320 factors, which are not required for the biogenesis of ISC but act specifically for transferring ISC to
321 mitochondrial target apoproteins [62]. By contrast, dysfunctions of the core mitochondrial ISC
322 assembly machinery induced a second pathway involving a Mec1p-independent activation of
323 Dun1p. Similarly, Sanvisens and colleagues [63] found that depletion of core components of the

mitochondrial ISC assembly activated a Dun1p-dependent but Mec1p- and Rad53p-independent pathway leading to increased dNTPs biosynthesis, needed for DNA repair.

Thus, Dun1p is a central actor in the activation of the DNA damage checkpoint induced by dysfunctions of the ISC assembly machineries [62]. Abbà and colleagues [19] found that Dun1p was necessary for the cadmium tolerant phenotype of FCR1 in a Mec1p-independent pathway. Here, we show that Dun1p was also required for the cadmium tolerant phenotype of Onzin-expressing yeast (Fig 5), suggesting that both PLAC8 proteins may activate a specific Dun1p-dependent DNA damage checkpoint pathway similar to the one described by Pijuan et al, [62] and Sanvisens et al, [63]. Indeed, Dun1p activates the RNR at multiple levels (i.e. phosphorylation of the Sml1p RNR1-inhibitor; phosphorylation and degradation of Dif1p, promoting the redistribution of the small RNR2 and RNR4 subunits to the cytoplasm where the catalytic RNR1 subunit resides; phosphorylation of the Crt1 transcriptional RNR2/3/4 repressor; Fig 5, [56]). Interestingly, the *PLAC8 down-regulated* mRNA-binding protein TIS11, belonging to the iron regulon, targets for degradation specific mRNAs encoding proteins that contain iron as a cofactor, including the small subunit of the ribonucleotide reductases (RNR2) as part of metabolic remodeling to conserve and optimize iron utilization [64].

Ancestral functions of PLAC8-containing proteins?

Proteins containing the PLAC8 domain are widely distributed in Eukaryotes and the PLAC8 proteins characterized so far are assigned two alternative functions, either in heavy metal resistance or as cell growth regulators. In this work, we showed that Onzin, the reference for PLAC8 cell growth regulators [2], could induce cadmium resistance in *S. cerevisiae*, demonstrating that these two functions are not mutually exclusive in PLAC8 proteins. Another example of how the same PLAC8 protein can display both functions is the rice OsPCR1 that, even though with different acronyms, has been reported as cell growth regulator [16] and able to confer cadmium-resistance [65].

350 Although the *S. cerevisiae* genome does not encode PLAC8 proteins, expression of FCR1 and
 351 Onzin induced ancient and highly conserved pathways that play a central role in cell growth and
 352 metabolism. In addition to iron homeostasis, components of the leucine biosynthetic pathway are
 353 involved in additional regulatory functions related to general cell growth and metabolism. The
 354 mitochondrial BCAA transaminase Bat1p is particularly interesting because it shows striking
 355 sequence similarity to the mammalian protein Eca39, a known target for c-Myc regulation [66, 67].
 356 Myc proteins are involved in cell proliferation and differentiation in vertebrates [68] and although
 357 they have not been found in yeast, a shortened G1 stage was observed in *bat1* yeast mutants [69].
 358 Thus, in addition to a role in BCAA biosynthesis and ISC translocation, Bat1p is also involved in
 359 cell cycle regulation. It is intriguing that Onzin, another known target of c-Myc activity in mammals
 360 [2, 70], increased cell survival and growth in the presence of cadmium by modulating the same
 361 conserved biosynthetic pathways.

362 Leucine biosynthesis was the main pathway up-regulated by both PLAC8 proteins in cadmium-
 363 exposed yeast. In addition to its interaction with iron homeostasis, this pathway generates the amino
 364 acid leucine, one of the most conserved TORC1 activating growth signals. TORC1 is a master
 365 regulator of cell proliferation, survival and growth found in yeast and mammals [50] and is
 366 activated through a pathway, partly conserved in Eukaryotes, that uses leucyl-tRNA synthetases as
 367 leucine sensors [71]. In yeast, this pathway regulates cellular responses to a variety of
 368 environmental stresses and is a determinant of cell survival in response to DNA damage, thanks to
 369 increased dNTPs synthesis [54]. It would be interesting to understand whether the PLAC8 and the
 370 TORC1 induced pathways may potentially interact in yeast.

371

372 In conclusion, our data clearly demonstrate that two PLAC8 proteins with different described
 373 functions in taxonomically distant organisms induced the same phenotype when expressed in *S.*
 374 *cerevisiae*, suggesting a common ancestral function. Having identified some processes and
 375 pathways regulated by both PLAC8 proteins, the two main functions ascribed to these proteins (i.e.

increased cadmium tolerance and regulation of cell proliferation) appear more related, as they could be both linked to leucine and ISC biosynthesis. Although this hypothesis requires further investigations, our results open new perspectives on the role of the PLAC8 protein domain in Eukaryotes and provide guidelines to explore in more details the exact role of the PLAC8 domain in the activation of important biological processes that ensure nuclear DNA integrity during cell division and metal stress responses.

Methods

Yeast strains and growth conditions

All yeast strains used in this work are listed in supplementary the S1 Appendix. EAY1269 wild type strain was kindly provided by Prof. Eric Alani (Cornell University, Ithaca, NY, USA). Yeast strains were grown at 30°C on Yeast Extract Peptone medium (YP) supplemented with 2% (W/v) glucose (YPD). Yeasts transformed with episomal plasmids were grown at 30°C on Yeast Nitrogen Base (YNB) medium supplemented with essential amino acids and either 2% (W/v) glucose (YNB-D) or 2% (W/v) galactose (YNB-Gal). All reagents were purchased from Sigma-Aldrich. Yeasts were transformed according to Gietz & Woods [72]. Transformation was confirmed by colony-PCR as described by Sambrook & Russell [73] with FL1 and FL2 primers (S2 Appendix). Strains transformed with pFL61-derived vectors were grown in medium lacking uracil. Yeasts co-transformed with pMS207 vector were grown in medium lacking leucine also. Media and growth conditions for Yeast Two Hybrid were prepared as previously described [19].

Protein sequences alignment

The protein alignment was performed using the Phylogeny.fr platform [74]. Sequences were aligned with MUSCLE (v3.8.31) configured for highest accuracy (MUSCLE with default settings).

401 **Spot dilution assay**

402 Spot dilution assays were used to monitor cell growth at various cadmium concentrations and were
 403 conducted as previously described [19], with the following modifications: for all conditions,
 404 overnight yeast cultures were diluted to OD₆₀₀=0.1 and cultured at 30°C until OD₆₀₀=0.2.
 405 Subsequently, 4 µl of serial dilutions (ranging from 5x10⁷ to 5x10² cells/ml) of each strain were
 406 spotted onto control or cadmium-amended YNB-D medium and incubated at 30°C for 4 days.

407

408 **Determination of cadmium half inhibitory concentration (IC₅₀)**

409 One clone of each EAY1269 yeast strain (transformed with the empty vector or expressing FCR1 or
 410 Onzin or Onzin^{Δ28-38}) was inoculated in 5 ml YNB-D medium and incubated at 30°C overnight. The
 411 following morning, each pre-inoculum was diluted in fresh YNB-D medium at optical density
 412 OD₆₀₀=0.1, and poured in 96-well plates. The cultures were amended with increasing concentrations
 413 CdSO₄ (from 0 to 400 µM), incubated at 30° C and 150 rpm, and the OD₆₀₀ was measured by using
 414 a microplate reader (TECAN) after 24 hours. The experiment was independently replicated 3 times,
 415 with 5 technical replicates per concentration and per clone (n=15). IC₅₀ values, representing the Cd
 416 concentration causing a 50% of inhibition of the yeast growth, were calculated using the ED50 Plus
 417 v1.0 available on line, and previously utilized and validated [75]. S1 Fig shows the distribution of
 418 the data from the three experiments, with standard deviation. Different letters indicated statistically
 419 different results (p<0.05). Single data from the three experiments, Shapiro Wilk test for normal
 420 distribution, p-values calculated by applying the ANOVA with Tukey as post-hoc test are are
 421 available in the S1 Data.

422

423 **Growth curve and cell viability assay**

424 For the growth curve assay, three clones of each EAY1269 yeast strain (transformed with the empty
 425 vector or expressing FCR1 or Onzin) were inoculated in 5 ml YNB-D medium and incubated at
 426 30°C overnight. The following morning, each pre-inoculum was diluted to 50 mL in fresh YNB-D

medium at optical density $OD_{600} = 0.1$, and split into two sterile flasks, one amended with 25 μM cadmium sulphate and the other left unamended. The optical density of cadmium-treated and control cultures was measured after 2, 4, 6, 8, 10, and 24h of incubation at 30°C. The data were tested for normal distribution with the Shapiro Wilk test ($p\text{-value} > 0.05$) and the ANOVA was applied with Tukey as post-hoc test. Single data and the calculated p-value for data at 6 and 8 hours are shown in the S2 Data. Fig 2B shows the result of a single experiment ($n=3$) that is representative of three independently repeated experiments, each one based on $n=3$ or $n=5$ biological replicates, and the raw data obtained after 8hrs of incubation are reported in the S3 Appendix. All the statistics were performed using PAST [76].

To evaluate the number of viable yeast cells during growth in liquid culture, the number of Colony Forming Units (CFU) was measured by plating 100 μL of the appropriate dilutions (based on the initial OD) onto standard YNB-D medium after 6, 8, 10 and 24 h of growth in control or cadmium-amended liquid YNB-D. Fig 2C reports the distribution of six biological replicates for the time-points 6, 10 and 24 h and three biological replicates for the 8h time-point, obtained in two independent experimental repetitions. Single data, Shapiro Wilk test for normal distribution, p-values calculated by applying the ANOVA with Tukey as post-hoc test are reported in the S3 Data.

443

444 **Onzin coding sequence isolation and expression in yeast**

445 All oligonucleotide sequences are listed in S2 Appendix. cDNA corresponding to *Mus musculus*
446 Onzin CDS was synthesized with Qiagen OneStep RT-PCR Kit (Qiagen, Venlo, Netherlands) using
447 total RNA from mouse blood and Not_Onzin1f/Not_Onzin2r primers which carry *NotI* restriction
448 site at the 5' end. PCR program was as follows: 60s at 98°C for 1 cycle; 10s at 94°C, 30s at 60°C,
449 40s at 72°C for 35 cycles; 10 min at 72° C for 1 cycle. Amplified DNA was digested with *NotI*
450 restriction enzyme and cloned into the pFL61 Vector. Sequence as well as direction of the inserted
451 gene were assessed by PCR and sequencing using primers FL1 and Not_Onzin2r. This vector was
452 named pFL61-Onzin. The mutant allele Onzin ^{$\Delta 28-38$} was obtained using a two-round mutagenesis

453 PCR. In the first round, the 5' end of the sequence was amplified with primers
454 Not_Onzin1f/Onzindel_r while the 3' end was amplified using primers Onzindel_f/Not_Onzin2r
455 using Thermo Phusion High Fidelity DNA Polymerase (Thermo, Waltham, MA, USA). PCR
456 program was as follows: 30s at 98°C for 1 cycle; 10s at 98°C, 30s at 62°C, 20s at 72°C for 35
457 cycles; 10 min at 72° C for 1 cycle. In the second round of PCR, the two purified products from the
458 first amplification were used as template for a fusion-PCR using primers Not_Onzin1f
459 /Not_Onzin2r and the same enzyme used before. PCR conditions were as follows: 30s at 98°C for 1
460 cycle; 10s at 98°C, 40s at 62°C, 20s at 72°C for 35 cycles; 10 min at 72° C. The PCR product was
461 then digested with the restriction enzyme *NotI*, ligated into the plasmid pFL61 previously digested
462 with the same enzyme and cloned into *E. coli*. Transformed colonies were screened by colony PCR
463 with primers FL1/Not_Onzin2r and positive plasmids were sequenced with primers FL1 and FL2 to
464 confirm the construct sequence. The pFL61-FCR1 construct has been obtained in a previous work
465 [19].

466

467 **Synthesis of EGFP-tagged Onzin construct and microscopy observations**

468 Construction of C-terminal Enhanced Green Fluorescent Protein (EGFP) tag of Onzin was
469 performed by amplifying the Onzin cDNA with primers HindIII_Onzin_f/XmaI_Onzin_r, carrying
470 *HindIII* and *XmaI* restriction sites and by removing the CDS stop codon. The DNA obtained was
471 then digested and directionally ligated in a previously digested (with the same enzymes) pEGFP-N1
472 vector and cloned into *E. coli*. Colony PCR with primers HindIII_Onzin_f/Not_EGFP_r was used
473 to identify and purify the right construct which was then confirmed by sequencing with the same
474 primers. To express Onzin-EGFP gene in yeast cells, the purified construct was used as template for
475 a second PCR with primers Not_Onzin1f/Not_EGFP_r and the PCR product was digested with *NotI*
476 enzyme, ligated in pFL61 plasmid previously digested with the same enzyme and cloned in *E. coli*.
477 Colony PCR using primers Not_Onzin1f / FL2 was used to confirm size and 5'-3' orientation of the

478 construct. This vector was called pFL61-Onzin-EGFP. The pFL61-FCR1-EGFP construct has been
479 obtained in a previous work [19].

480 Yeast cells expressing an inducible tomato protein carrying a nuclear localization signal (NLS)-RFP
481 (plasmid pMS207, courtesy of prof. Maya Schouldiner, Weizmann Institute, Rehovot, Israel) were
482 transformed either with the plasmid carrying the constitutive Onzin-EGFP tagged construct (pFL61-
483 Onzin-EGFP) or with the plasmid carrying the FCR1-EGFP tagged construct. Double transformants
484 were grown overnight with galactose as the sole carbon source (YNB-Gal). The localization of
485 EGFP-tagged and RFP-tagged proteins was observed on a Leica TCS SP2 confocal microscope,
486 using a long-distance 40X water-immersion objective (HCX Apo 0.80). For EGFP visualization, an
487 Argon laser band of 488nm was used for excitation and the emission window was recorded between
488 500 and 525nm, while for the RFP-tagged protein a laser light of 554 nm was used, and an emission
489 window of 581 nm.

490

491 **Yeast-Two-Hybrid**

492 The yeast two-hybrid assay was performed using the DupLEX-A yeast system (Origene
493 Technologies, Rockville, MD, USA) as described in previous work [19]. The MmOnzin coding
494 sequence was cloned in frame with the DNA binding domain of LexA into the pEG202 vector using
495 primers Eco_MmOnzin_f/Not_Onzin2r. The C-terminal region of *MLH3* from *Mus musculus* was
496 isolated with Qiagen OneStep RT-PCR Kit (Qiagen, Venlo, The Netherlands) using total RNA from
497 mouse blood and primers Eco_MmMLH3_f/Eco_MmMLH3_r (S2 Appendix) then cloned
498 downstream in frame with the activator domain of B42 into the pEG202 vector. Sequence and
499 orientation were confirmed by colony-PCR and Sanger sequencing. All the other vectors and strains
500 used in the yeast-two-hybrid assay were generated in previous work [19].

501

Lys⁺ reversion assay

EAY1269 strains (transformed with the empty vector or expressing FCR1 or Onzin) were analyzed for reversion to the *Lys⁺* phenotype according to Tran et al, [25]. Cells were cultured in YNB-D for 24 hrs at 30°C and 150 rpm shaking with or without 1μM CdSO₄, a concentration that did not influence cell growth in 24 hrs. At the end of the incubation, 100 μl of the appropriate dilution were plated on normal and *Lys⁻* dropout medium. Colonies were counted after four days. At least n=20 independent biological replicates for each strain and condition were analyzed (reported in Table 1). Reversion rates were determined as previously described [77]. Confidence intervals of 95% were determined as described by [78]. Each median reversion rate was normalized to the empty vector median rate to calculate the fold increase in mutation rate.

RNAseq analysis

EAY1269 cells expressing Onzin, FCR1 or transformed with the empty vector (pFL61) were grown in three biological replicates (n=3), as described for the growth curve, on YNB-D with 25 μM CdSO₄. After 8hrs, cells were harvested by centrifugation, washed with sterile water and frozen in liquid nitrogen. Total RNA was extracted in a CTAB-based extraction buffer (2% CTAB, 2% PVP, 100 mM Tris-HCl pH 8, 25 mM EDTA, 2 M NaCl, 2% β-mercaptoethanol, 1% (W/v) PVPP). The homogenates were incubated 5 min at 65°C, extracted twice in chloroform:isoamyl alcohol 24:1 (v/v), precipitated with an equal volume of LiCl 10M (on-ice over-night precipitation), resuspended in an SSTE buffer (1 M NaCl, 0.5% (W/v) SDS, 10 mM Tris-HCl pH 8, 1 mM EDTA), extracted with phenol:chloroform:isoamyl alcohol 25:24:1 (v/v/v), extracted in chloroform:isoamyl alcohol 24:1 (v/v), precipitated with 100% ethanol (2 hrs, -20°C), washed in 80% ethanol and resuspended in DEPC-treated water. The quantity and quality of the extracted RNA was evaluated with a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The mRNA was sequenced by Illumina technology with Hiseq 2000, raw reads of 50 NTs were firstly assessed for their quality using FastQC and then mapped on *S. cerevisiae* S288C genome with Burrows-Wheeler Aligner

[79]. Resulting BAM files were processed using SAMtools [80]. Only reads with phred score greater than 15 were considered in the analysis. The genome sequence and annotation files were obtained from SGD database (<http://www.yeastgenome.org>). The differential gene expression was assessed with DESeq package [81], retaining only genes with FDR- corrected p-value <0.1. Genes with read counts equal to zero in all replicates of at least one experimental condition were excluded from the analysis. The fold change was calculated with respect to the empty-vector expressing strain and genes with log₂ fold-change >1 or <-1 and with adjusted p-value <0.05 were considered respectively up- or down-regulated by the PLAC8 proteins. Gene Ontology and pathway enrichment were evaluated using DAVID Bioinformatics Resources 6.8 and Saccharomyces Genome Database [82, 83].

538

539 **Respiratory activity**

Yeast cells were cultured over night at 28°C in YNB medium supplemented with 0.6% glucose, then cells were treated for 4 hrs with up to 200µM CdSO₄. Oxygen consumption rate was measured at 30°C using a Clark-type oxygen electrode (Oxygraph System Hansatech Instruments England) with 1 mL of air-saturated respiration buffer (0.1 M phthalate–KOH, pH 5.0), 0.5% glucose. The experiment was independently performed twice (n=2), with one biological replicate per sample.

545

546 **Metal content analysis**

The EAY1269 yeast strains, transformed with the empty vector or expressing either FCR1 or Onzin, were inoculated in 5 ml YNB-D medium and incubated at 30°C overnight. The following morning, each pre-inoculum was diluted to 50 mL of the control YNB-D medium, or in the same medium supplemented with 25 µM CdSO₄. Five different clones were used for each transformant, per each experimental condition (n=5). Yeast cultures were harvested after 8h and centrifuged at 4000 rpm for 5 min. Cells were washed three times with 10 mM EDTA in 50 mM Tris–HCl buffer (pH 6.5), and with milli-Q water. Finally, samples were dried at 60 °C for 2 days, and subsequently

mineralized with 1 ml HNO₃ 6M in a bath at 90°C for 1h. After dilution to a final concentration of 1M HNO₃, the metal content was determined using Induced Coupled Plasma (ICP-OES Optima 7000 DV, Perkin Elmer). Controls made up of milli-Q water and nitric acid 1M. Statistics have been performed by using the Past software [76]. Normality of data was assessed by Shapiro Wilk test. Pairwise differences have been calculated by the Mann-Whitney test for differences in the medians. Different letters indicate significant differences between samples (p<0.05). The raw data and single p-values are reported in the S4 Data.

Data Availability

All relevant data are within the manuscript and its Supporting Information files. The RNAseq data from this publication have been deposited to the SRA database, have been assigned the identifier SRP145576 and will be available after acceptance.

Acknowledgements

We thank Simona Abbà (Institute for Sustainable Plant Protection of the National Research Council of Italy, IPSP-CNR, Turin, Italy) for Onzin gene isolation and support through the project, Eric Alani (Cornell University, Ithaca, NY, USA) for providing the EAY1269 strain and technical support with the Lys2⁺ reversion assay, and Maya Schuldiner (Weizmann Institute of Sciences, Rehovot, Israel) for the pMS207 vector and technical support in the FCR1 and Onzin cellular localization.

576 **References**

- 577 1. Galaviz-Hernandez C, Stagg C, de Ridder G, Tanaka TS, Ko MSH, Schlessinger D, et al. Plac8
578 and Plac9, novel placental-enriched genes identified through microarray analysis. *Gene*.
579 2003;309(2): 81-89.
- 580 2. Rogulski K, Li Y, Rothermund K, Pu L, Watkins S, Yi F, Prochownik EV. Onzin, a c-Myc-
581 repressed target, promotes survival and transformation by modulating the Akt-Mdm2-p53 pathway.
582 *Oncogene*. 2005;24(51): 7524–7541.
- 583 3. Wu SF, Huang Y, Hou JK, Yuan TT, Zhou CX, Zhang J, et al. The downregulation of onzin
584 expression by PKC epsilon-ERK2 signaling and its potential role in AML cell differentiation.
585 *Leukemia*. 2010;24(3): 544–551.
- 586 4. Kaistha BP, Lorenz H, Schmidt H, Sipos B, Pawlak M, Gierke B, et al. PLAC8 localizes to the
587 inner plasma membrane of pancreatic cancer cells and regulates cell growth and disease progression
588 through critical cell-cycle regulatory pathways. *Cancer Res*. 2016;76(1): 96-107.
- 589 5. Zou L, Chai J, Gao Y, Guan J, Liu Q, Du JJ. Down-regulated PLAC8 promotes hepatocellular
590 carcinoma cell proliferation by enhancing PI3K/Akt/GSK3 β /Wnt/ β -catenin signaling. *Biomed*
591 *Pharmacother*. 2016;84: 139-146.
- 592 6. Lee CL, Huang CJ, Yang SH, Chang CC, Huang CC, Chien CC, et al. Discovery of genes from
593 feces correlated with colorectal cancer progression. *Oncology Lett*. 2016;12(5): 3378-3384.
- 594 7. Li C, Ma H, Wang Y, Cao Z, Graves-Deal R, Powell AE, et al. Excess PLAC8 promotes an
595 unconventional ERK2-dependent EMT in colon cancer. *J Clin Invest*. 2014;124(5): 2172-2187.
- 596 8. Jimenez-Preitner M, Berney X, Thorens B. Plac8 is required for white adipocyte differentiation
597 *in vitro* and cell number control *in vivo*. *PLoS One*. 2012;7(11): e48767.
- 598 9. Mourtada-Maarabouni M, Watson D, Munir M, Farzaneh F, Williams GT. Apoptosis suppression
599 by candidate oncogene PLAC8 is reversed in other cell types. *Curr Cancer Drug Targets*.
600 2013;13(1): 80-91.

- 601 10. Cong B, Tanksley SD. FW2.2 and cell cycle control in developing tomato fruit: a possible
602 example of gene co-option in the evolution of a novel organ. *Plant Mol Biol.* 2006;62(6): 867-880.
- 603 11. Dahan Y, Rosenfeld R, Zadiranov V, Irihimovitch V. A proposed conserved role for an avocado
604 FW2.2-like gene as a negative regulator of fruit cell division. *Planta.* 2010;232 (3): 663-676.
- 605 12. De Franceschi P, Stegmeir T, Cabrera A, van der Knaap E, Rosyara UR, Sebolt AM, et al. Cell
606 number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and
607 sour cherry. *Mol Breed.* 2013;32 (2): 311-326.
- 608 13. Li Z, He C. *Physalis floridana* Cell Number Regulator1 encodes a cell membrane-anchored
609 modulator of cell cycle and negatively controls fruit size. *J Exp Bot.* 2015;66 (1): 257-270.
- 610 14. Tian J, Zeng B, Luo S, Li X, Wu B, Li J. Cloning, localization and expression analysis of two
611 fw2.2-like genes in small- and large-fruited pear species. *J Integr Agric.* 2016;15(2): 282-294.
- 612 15. Guo M, Rupe MA, Dieter JA, Zou J, Spielbauer D, Duncan KE, et al. Cell Number Regulator1
613 affects plant and organ size in maize: implications for crop yield enhancement and heterosis. *Plant*
614 *Cell.* 2010;22(4): 1057-1073.
- 615 16. Xu J, Xiong W, Cao B, Yan T, Luo T, Fan T, et al. Molecular characterization and functional
616 analysis of "fruit-weight 2.2-like" gene family in rice. *Planta.* 2013;238(4): 643-655.
- 617 17. Song WY, Martinoia E, Lee J, Kim D, Kim DY, Vogt E, et al. A novel family of cys-rich
618 membrane proteins mediates cadmium resistance in *Arabidopsis*. *Plant Physiol.* 2004;135(2):1027-
619 1039.
- 620 18. Song WY, Choi KS, Kim DY, Geisler M, Park J, Vincenzetti V, et al. *Arabidopsis* PCR2 is a
621 zinc exporter involved in both zinc extrusion and long-distance zinc transport. *Plant Cell.*
622 2010;22(7): 2237-2252.
- 623 19. Abbà S, Vallino M, Daghino S, Di Vietro L, Borriello R, Perotto S. A PLAC8-containing
624 protein from an endomycorrhizal fungus confers cadmium resistance to yeast cells by interacting
625 with Mlh3p. *Nucleic Acids Res.* 2011;39 (17): 7548-7563.

- 626 20. Di Vietro L, Daghino S, Abbà S, Perotto S. Gene expression and role in cadmium tolerance of
627 two PLAC8-containing proteins identified in the ericoid mycorrhizal fungus *Oidiodendron maius*.
628 Fungal Biol. 2014;118(8): 695-703.
- 629 21. Hartwig A. Mechanisms in cadmium-induced carcinogenicity: recent insights. *Biometals*. 2010;
630 23(5): 951-960.
- 631 22. Jin YH, Clark AB, Slebos RJ, Al-Refai H, Taylor JA, Kunkel TA, et al. Cadmium is a mutagen
632 that acts by inhibiting mismatch repair. *Nat Genet*. 2003;34(3): 326-329.
- 633 23. Giaginis C, Gatzidou E, Theocharis S. DNA repair systems as targets of cadmium toxicity.
634 *Toxicol Appl Pharmacol*. 2006;213(3): 282-290.
- 635 24. Flores-Rozas H, Kolodner RD. The *Saccharomyces cerevisiae* MLH3 gene functions in MSH3-
636 dependent suppression of frameshift mutations. *Proc Natl Acad Sci USA*. 1998;95(21): 12404-
637 12409.
- 638 25. Tran HT, Keen JD, Krickler M, Resnick MA, Gordenin DA. Hypermutability of homonucleotide
639 runs in mismatch repair and DNA polymerase proofreading yeast mutants. *Mol Cell Biol*.
640 1997;17(5): 2859-2865.
- 641 26. Greene CN, Jinks-Robertson S. Frameshift intermediates in homopolymer runs are removed
642 efficiently by yeast mismatch repair proteins. *Mol Cell Biol*. 1997;17(5): 2844-2850.
- 643 27. Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, et al. Cadmium stress:
644 an oxidative challenge. *Biometals*. 2010;23(5):927-940.
- 645 28. Singh S, Brocker C, Koppaka V, Chen Y, Jackson BC, Matsumoto A, et al. Aldehyde
646 dehydrogenases in cellular responses to oxidative/electrophilic stress. *Free Radic Biol Med*.
647 2013;56: 89-101.
- 648 29. Kurita O, Nishida Y. Involvement of mitochondrial aldehyde dehydrogenase ALD5 in
649 maintenance of the mitochondrial electron transport chain in *Saccharomyces cerevisiae*. *FEMS*
650 *Microbiol Lett*. 1999;181(2): 281-287.

- 651 30. Marobbio CM, Giannuzzi G, Paradies E, Pierri CL, Palmieri F. alpha-Isopropylmalate, a leucine
652 biosynthesis intermediate in yeast, is transported by the mitochondrial oxalacetate carrier. *J Biol*
653 *Chem.* 2008;283(42): 28445-28453.
- 654 31. Kohlhaw GB. Leucine biosynthesis in fungi: entering metabolism through the back door.
655 *Microbiol Mol Biol Rev.* 2003;67(1): 1-15.
- 656 32. Robertson LS, Causton HC, Young RA, Fink GR. The yeast A kinases differentially regulate
657 iron uptake and respiratory function. *Proc Natl Acad Sci USA.* 2000;97: 5984– 5988.
- 658 33. Richardson DR, Lane DJ, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, et al.
659 Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion
660 and cytosol. *Proc Natl Acad Sci USA.* 2010;107(24): 10775-10782.
- 661 34. Lill R, Mühlenhoff U. Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected
662 processes, and diseases. *Annu Rev Biochem* 2008;77: 669–700.
- 663 35. Lill R. Function and biogenesis of iron-sulphur proteins. *Nature.* 2009;460: 831-838.
- 664 36. Stehling O, Lill R. The role of mitochondria in cellular iron-sulfur protein biogenesis:
665 mechanisms, connected processes, and diseases. *Cold Spring Harb Perspect Biol.* 2013;5(8):
666 a011312.
- 667 37. Maio N, Rouault TA. Iron–sulfur cluster biogenesis in mammalian cells: new insights into the
668 molecular mechanisms of cluster delivery. *Biochim Biophys Acta.* 2015;1853(6): 1493-1512.
- 669 38. Gerber J, Neumann K, Prohl C, Mühlenhoff U, Lill R. The yeast scaffold proteins Isu1p and
670 Isu2p are required inside mitochondria for maturation of cytosolic Fe/S proteins. *Mol Cell Biol.*
671 2004;24(11): 4848-4857.
- 672 39. Zhang Y, Lyver ER, Nakamaru-Ogiso E, Yoon H, Amutha B, Lee DW, et al. Dre2, a conserved
673 eukaryotic Fe/S cluster protein, functions in cytosolic Fe/S protein biogenesis. *Mol Cell Biol*
674 2008;28(18): 5569-5582.

675 40. Bedekovics T, Li H, Gajdos GB, Isaya G. Leucine biosynthesis regulates cytoplasmic iron-
676 sulfur enzyme biogenesis in an Atm1p-independent manner. J Biol Chem. 2011;286(47): 40878-
677 40888.

678 41. Ihrig J, Hausmann A, Hain A, Richter N, Hamza I, Lill R, et al. Iron regulation through the back
679 door: iron-dependent metabolite levels contribute to transcriptional adaptation to iron deprivation in
680 *Saccharomyces cerevisiae*. Eukaryot Cell. 2010;9: 460-471.

681 42. Rouault TA. The role of iron regulatory proteins in mammalian iron homeostasis and disease.
682 Nat Chem Biol. 2006;2: 406-414.

683 43. Philpott CC, Leidgens S, Frey AG. Metabolic remodeling in iron-deficient fungi. Biochim
684 Biophys Acta. 2012;1823(9): 1509-1520.

685 44. Ruotolo R, Marchini G, Ottonello S. Membrane transporters and protein traffic networks
686 differentially affecting metal tolerance: a genomic phenotyping study in yeast. Genome Biol
687 2008;9(4): R67.

688 45. Thorsen M, Perrone GG, Kristiansson E, Traini M, Ye T, Dawes IW, et al. Genetic basis of
689 arsenite and cadmium tolerance in *Saccharomyces cerevisiae*. BMC Genomics 2009;10: 105.

690 46. Lesuisse E, Labbe P. Effects of cadmium and of YAP1 and CAD1/YAP2 genes on iron
691 metabolism in the yeast *Saccharomyces cerevisiae*. Microbiology. 1995;141: 2937-2943.

692 47. Momose Y, Iwahashi H. Bioassay of cadmium using a DNA microarray: genome-wide
693 expression patterns of *Saccharomyces cerevisiae* response to cadmium. Environ Toxicol Chem.
694 2001;20(10): 2353-2360.

695 48. Chen OS, Crisp RJ, Valachovic M, Bard M, Winge DR, Kaplan J. Transcription of the yeast
696 iron regulon does not respond directly to iron but rather to iron-sulfur cluster biosynthesis. J Biol
697 Chem. 2004;279, 29513-29518.

698 49. Ueta R, Fujiwara N, Iwai K, Yamaguchi-Iwai Y. Iron-induced dissociation of the Aft1p
699 transcriptional regulator from target gene promoters is an initial event in iron-dependent gene
700 suppression. Mol Cell Biol. 2012;32: 4998-5008.

701 50. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. Trends Cell Biol. 2014;24:
702 400-406.

703 51. Kingsbury JM, Sen ND, Cardenas ME. Branched-chain aminotransferases control TORC1
704 signaling in *Saccharomyces cerevisiae*. PLoS Genet. 2015;11: e1005714.

705 52. Fournier ML, Paulson A, Pavelka N, Mosley AL, Gaudenz K, Bradford WD, et al. Delayed
706 correlation of mRNA and protein expression in rapamycin-treated cells and a role for Ggc1 in
707 cellular sensitivity to rapamycin. Mol Cell Proteomics. 2010;9.2: 271-284.

708 53. Lesuisse E, Lyver ER, Knight SAB, Dancis A. Role of YHM1, encoding a mitochondrial carrier
709 protein, in iron distribution of yeast. Biochem. J 2004;378: 599-607.

710 54. Shen C, Lancaster CS, Shi B, Guo H, Thimmaiah P, Bjornsti MA. TOR signaling is a
711 determinant of cell survival in response to DNA damage. Mol Cell Biol. 2007;27(20): 7007-7017.

712 55. White MF, Dillingham MS. Iron-sulphur clusters in nucleic acid processing enzymes. Curr Opin
713 Struct Biol. 2012;22: 94-100.

714 56. Puig S, Ramos-Alonso L, Romero AM, Martinez-Pastor MT. The elemental role of iron in
715 DNA synthesis and repair. Metallomics. 2017;9: 1483-1500.

716 57. Arnold AR, Grodick MA, Barton JK. DNA Charge Transport: from chemical principles to the
717 cell. Cell Chem Biol. 2016;23(1): 183-197.

718 58. Zhang C. Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle
719 control. Protein Cell 2014;5(10), 750-760.

720 59. Paul VD, Lill R. Biogenesis of cytosolic and nuclear iron–sulfur proteins and their role in
721 genome stability. Biochim Biophys Acta. 2015;1853: 1528-1539.

722 60. Guo L, Ganguly A, Sun L, Suo F, Du LL, Russell P. Global fitness profiling identifies Arsenic
723 and Cadmium Tolerance Mechanisms in Fission Yeast. G3. 2016;6(10): 3317-3333.

724 61. Zhou Z, Elledge SJ. DUN1 encodes a protein kinase that controls the DNA damage response in
725 yeast. Cell 1993;75(6): 1119-1127.

726 62. Pijuan J, María C, Herrero E, Belli G. Impaired mitochondrial Fe-S cluster biogenesis activates
727 the DNA damage response through different signaling mediators. *J Cell Sci.* 2015;128(24): 4653-
728 4665.

729 63. Sanvisens N, Romero AM, Zhang C, Wu X, An X, Huang M, et al. Yeast Dun1 kinase regulates
730 ribonucleotide reductase small subunit localization in response to iron deficiency. *J Biol Chem.*
731 2014;291(18): 9807-9817.

732 64. Puig S, Askeland Thiele DJ. Coordinated remodeling of cellular metabolism during iron
733 deficiency through targeted mRNA degradation. *Cell.* 2005;120: 99-110.

734 65. Wang FJ, Wang M, Liu ZP, Shi Y, Han TQ, Ye YY, et al. Different responses of low grain-Cd-
735 accumulating and high grain-Cd-accumulating rice cultivars to Cd stress. *Plant Physiol Bioch.*
736 2015;96: 261-269.

737 66. Kispal G, Steiner H, Court DA, Rolinski B, Lill R. Mitochondrial and cytosolic branched-chain
738 amino acid transaminases from yeast, homologs of the myc oncogene-regulated Eca39 protein. *J*
739 *Biol Chem.* 1996;271(40): 24458-24464.

740 67. Eden A, Simchen G, Benvenisty N. Two yeast homologs of ECA39, a target for c-Myc
741 regulation, code for cytosolic and mitochondrial branched-chain amino acid aminotransferases. *J*
742 *Biol Chem.* 1996;271: 20242-20245.

743 68. Eilers M, Eisenman RN. Myc's broad reach. *Genes Dev.* 2008;22(20): 2755-2766.

744 69. Schuldiner O, Eden A, Ben-Yosef T, Yanuka O, Simchen G, Benvenisty N. ECA39, a
745 conserved gene regulated by c-Myc in mice, is involved in G1/S cell cycle regulation in yeast. *Proc*
746 *Natl Acad Sci USA.* 1996;93(14): 7143-7148.

747 70. Li Y, Rogulski K, Zhou Q, Sims PJ, Prochownik EV. The negative c-Myc target onzin affects
748 proliferation and apoptosis via its obligate interaction with phospholipid scramblase 1. *Molec Cell*
749 *Biol.* 2006;26(9): 3401–3413.

750 71. Gonzalez A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. *EMBO J.*
751 2017;36(4): 397-408.

752 72. Gietz RD, Woods RA. Yeast transformation by the LiAc/SS carrier DNA/PEG method.
753 Methods Mol Biol. 2006;313: 107-120.

754 73. Sambrook J, Russell DW. Analyzing yeast colonies by PCR. CSH Protoc. 2006(1). pii:
755 pdb.prot4015.

756 74. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust
757 phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36: W465-469.

758 75. Offman MN, Krol M, Patel N, Krishnan S, Liu J, Saha V et al. Rational engineering of L-
759 asparaginase reveals importance of dual activity for cancer cell toxicity. Blood. 2001;117: 1614-
760 1621.

761 76. Hammer Ø, Harper DAT, Ryan PD. PAST: Paleontological statistics software package for
762 education and data analysis. Palaeontologia Electronica. 2001;4(1): 9pp.

763 77. Heck JA, Gresham D, Botstein D, Alani E. Accumulation of recessive lethal mutations in
764 *Saccharomyces cerevisiae* mlh1 mismatch repair mutants is not associated with gross chromosomal
765 rearrangements. Genetics. 2006;174(1): 519-523.

766 78. Dixon WJ, Massey FJ Jr. *Introduction to Statistical Analysis*. 3rd ed. McGraw-Hill, New York,
767 USA; 1969.

768 79. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform.
769 Bioinformatics. 2010;26(5): 589-595.

770 80. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. 1000 Genome Project Data
771 Processing Subgroup. The Sequence Alignment/Map Format and SAMtools. Bioinformatics.
772 2009;25(16):2078-9.

773 81. Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol.
774 2010;11: R106.

775 82. Sherman BT, Huang da W, Tan Q, Guo Y, Bour S, Liu D, et al. DAVID Knowledgebase: a
776 gene-centered database integrating heterogeneous gene annotation resources to facilitate high-
777 throughput gene functional analysis. BMC Bioinformatics. 2007;8 (1): 426.

- 778 83. Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, et al.
779 Saccharomyces Genome Database: the genomics resource of budding yeast. Nucleic Acids Res.
780 2012;40: D700-D705.
- 781 84. Sonntag Brown M, Lim E, Chen C, Nishant KT, Alani E. Genetic analysis of mlh3 mutations
782 reveals interactions between crossover promoting factors during meiosis in baker's yeast. G3.
783 2013;3(1): 9-22.

784 **Figure captions**

785 **Fig 1. Amino acid sequence alignment of PLAC8 domain-containing proteins.**

786 The complete sequences of MmOnzin (from *Mus musculus*), HsOnzin (from *Homo sapiens*) and
787 OmFCR1 (from *Oidiodendron maius*) have been aligned. Similar residues are colored as the most
788 conserved one according to BLOSUM62 average scores: Max: 3.0 (light blue), Low: 0.5 (grey).
789 Lower case non-colored letters indicate amino acid residues with no similarities. The protein
790 alignment was performed using the Phylogeny.fr platform. The red box shows the PLAC8 domain.

792 **Fig 2. Growth and viability of yeast cells expressing FCR1 and Onzin on cadmium-containing media.**

794 (A) Spot dilution assay of yeast (EAY1269 strain) expressing FCR1, wild-type Onzin, the truncated
795 Onzin^{Δ28-38} or the empty vector pFL61 (EV). Strains were plated in ten-fold serial dilutions onto
796 YNB-D medium amended with CdSO₄ (10 or 25 μM) or not (control medium). (B) Growth curves
797 of yeast cell cultures in control medium (CTRL) or in a medium containing 25 μM CdSO₄ (Cd).
798 The optical density (OD₆₀₀) of cultures expressing FCR1, Onzin and pFL61 (EV) was measured
799 after 2-4-6-8-10-24 hours of incubation at 30°C and 150 rpm. The asterisks indicate time points
800 with a significant difference (n=3, Shapiro Wilk as normality test, ANOVA with Tukey P<0.01)
801 between cells expressing FCR1, or Onzin, and cells transformed with the empty vector (EV). (C)
802 Cell viability of yeasts expressing FCR1, Onzin or the empty vector pFL61 (EV) after growth for 6-
803 8-10-24 hours in control medium (CTRL) or in a medium containing 25 μM CdSO₄ (Cd). Colony
804 Forming Units (CFUs) were counted for each yeast culture. Samples showing statistically different
805 CFU numbers (P<0.05 by ANOVA with Tukey as post-hoc test, n=6 for 6-10-24h time points, n=3
806 for the 8h time point, Shapiro Wilk as normality test) are indicated by different letters. The square
807 symbol indicates the mean value. The whiskers indicate the minimum and the maximum values. The
808 top and the bottom of the rectangle indicate ± standard deviation, while the central line of the
809 rectangle indicates the 50%.

810

811 **Fig 3. Subcellular localization of FCR1 and Onzin proteins in yeast cells.**

812 The FCR1-GFP and the Onzin-GFP fusion proteins were localized to the yeast nucleus, as indicated
813 by co-localization (Merge) with a fusion protein carrying a nuclear localization signal (NLS)-RFP.
814 BF: bright field image of the yeast cells. Scale bar is 5µm.

815

816 **Fig 4. Schematic representation of the cellular functions of the PLAC8 up-regulated genes.**

817 PLAC8 up-regulated genes are indicated in orange. Genes for which functional assays were
818 performed are colored in green. Reactions catalyzed by proteins encoded by PLAC8 up-regulated
819 genes are indicated as full arrows. Dotted arrows represent processes or regulatory pathways known
820 from the literature (see text for references). Hexagons represent enzymatic complexes, whereas gray
821 ovals represent membrane carriers. ALD5: Aldehyde Dehydrogenase 5, BAT1: mitochondrial
822 BCAT, CIA: cytosolic ISC assembly, DIC1: Dicarboxylate Carrier 1, DRE2: Fe-S-containing
823 protein supplying reducing equivalents to the early steps of the cytosolic Fe-S assembly (CIA)
824 pathway, DUN1-P: DNA-damage uninducible kinase, in the phosphorylated active form, ETC:
825 electron transfer chain, GDH1: NADP(+)-dependent glutamate dehydrogenase, GGC1: GDP/GTP
826 Carrier 1, IAM: mitochondrial ISC assembly machinery, ILV2: acetolactate synthase, ILV3:
827 dihydroxiacid dehydratase, ILV5: acetohydroxiacid reductoisomerase, ISU2: mitochondrial protein
828 required for iron-sulfur protein synthesis, α -IPM: α -isopropylmalate, pyr: pyruvate, kb: α -
829 ketobutanoate, kiv: α -ketoisovalerate, kmv: α -ketomethylvalerate, LEU1: isopropyl malate
830 isomerase, LEU2: β -IPM dehydrogenase, LEU3: LEUcine biosynthesis transcription factor, acts as
831 an activator in the presence of α -isopropylmalate, LEU4: α -isopropylmalate synthase, LEU9: α -
832 isopropylmalate synthase (paralog of LEU4), MMR: DNA mismatch repair complex, OAC1:
833 OxaloAcetate Carrier 1, ODC2: OxoDicarboxylate Carrier 2, ORT1: ORnithine Transporter 1,
834 RNRC: ribonucleotide reductases complex, TORC1: target of rapamycin complex 1.

835

836 **Fig 5. Influence of DUN1 deletion on cadmium tolerance in yeast cells expressing FCR1 and**
 837 **Onzin.**

838 Spot dilution assay of wild-type W303 (WT) and *dun1* mutant strains expressing FCR1, Onzin or
 839 the empty vector pFL61 (EV). Strains were plated in ten-fold serial dilutions onto YNB-D medium,
 840 with or without 10 μ M CdSO₄.

841 **Supporting information captions**

842 **S1 Fig. Half Inhibitory Concentration (IC₅₀) of CdSO₄.**

843 Yeast expressing FCR1, wild-type Onzin, truncated Onzin^{Δ28-38} or the empty vector pFL61 (EV)
844 were exposed to increasing Cd concentrations and IC₅₀ have been determined. The distribution of
845 the data from three independent experiments is shown in the figure. The square symbol indicates the
846 mean value. The whiskers indicate the minimum and the maximum values. The top and the bottom of
847 the rectangle indicate $\pm$ standard deviation, while the central line of the rectangle indicates the 50%.
848 Statistically different results are indicated with different letters (P<0.05 by ANOVA with Tukey as
849 post-hoc test, Shapiro Wilk as normality test).

850

851 **S2 Fig. Yeast-Two-Hybrid to investigate FCR1 and Onzin interactions with different Mlh3** 852 **proteins.**

853 Genes coding for Mlh3p were isolated from *S. cerevisiae* (ScMlh3) from *O. maius* (OmMlh3) and
854 from *Mus musculus* (MmMlh3). Yeasts were plated with ten-fold dilutions onto galactose and β -
855 galactosidase containing-medium lacking leucine. The dark blue color of FCR1/ScMlh3 and
856 FCR1/OmMlh3 colonies indicates a strong protein–protein interaction. Onzin is able to interact
857 with both ScMlh3 and MmMlh3, but the blue color is less intense. No self-activation was observed
858 when cells were co-transformed with a vector expressing an irrelevant protein such as *O. maius*
859 superoxide dismutase (OmSOD1), that was used as negative control. A and B represent two
860 independent replicates of the same experiment.

861

862 **S3 Fig. Genes differentially regulated by PLAC8 proteins in the transcriptome of yeast cells** 863 **exposed to cadmium.**

864 The diagram shows the number of genes differentially regulated by Onzin (Onzin-regulated), by
865 FCR1 (FCR1-regulated) or by both proteins (PLAC8 regulated) in yeast cultures grown for 8 h in
866 cadmium-containing medium (25 μ M), as compared to the yeast strain transformed with the empty

867 vector.

868

869 **S4 Fig. Oxygen consumption rate of yeast strains expressing FCR1, Onzin or the empty**

870 **vector.**

871 Yeast cells were exposed to different CdSO₄ concentrations, ranging from 0 to 200 µM. The data

872 from two independent assays are plotted.

873

874 **S1 Table. Regulation of gene expression in yeast cells exposed to cadmium and expressing**

875 **FCR1 or Onzin.**

876 Sheet A: FCR1 up-regulated yeast genes; sheet B: Onzin up-regulated yeast genes; sheet C: FCR1

877 down-regulated yeast genes; sheet D: Onzin down-regulated yeast genes. PLAC8-regulated genes

878 are highlighted in red. The fold change was calculated with respect to the empty-vector expressing

879 strain and genes with log₂ fold-change >1 or <-1 and with adjusted p-value <0.05 were considered

880 respectively up- or down-regulated.

881

882 **S2 Table. GO categories and KEGG pathways enriched in PLAC8-regulated genes.**

883 Categories enriched with p-value<0.05 are reported.

884

885 **S3 Table. Iron and cadmium content in Cd-treated yeasts expressing Onzin, FCR1 or the**

886 **empty vector.**

887 Intracellular content of iron and cadmium in yeast cells exposed to cadmium (25 µM for 8h) was

888 measured after total biomass digestion by ICP-OES. Different letters indicate significant differences

889 between samples (p<0.05 according to Mann-Whitney test for differences in the medians with n=5).

890

891 **S1 Appendix. Yeast strains used in this work.**

892

893 **S2 Appendix. Yeast growth of unexposed and cadmium-exposed (Cd) cell cultures.**

894 The optical density (OD 600nm) of yeast cultures expressing FCR1, Onzin and the empty vector
 895 pFL61 (EV) was measured after growth in control medium or in Cd-containing medium (25uM) for
 896 8 hrs at 30°C, 150 rpm. The table reports the raw data for three independent experiments, each one
 897 including n=3 or n=5 biological replicate of each sample.

898

899 **S3 Appendix. List of oligonucleotides used in this work.**

900

901 **S1 Data. Source data and detailed statistics for S1 Fig.**

902

903 **S2 Data. Source data and detailed statistics for Fig 2B.**

904

905 **S3 Data. Source data and detailed statistics for Fig 2C.**

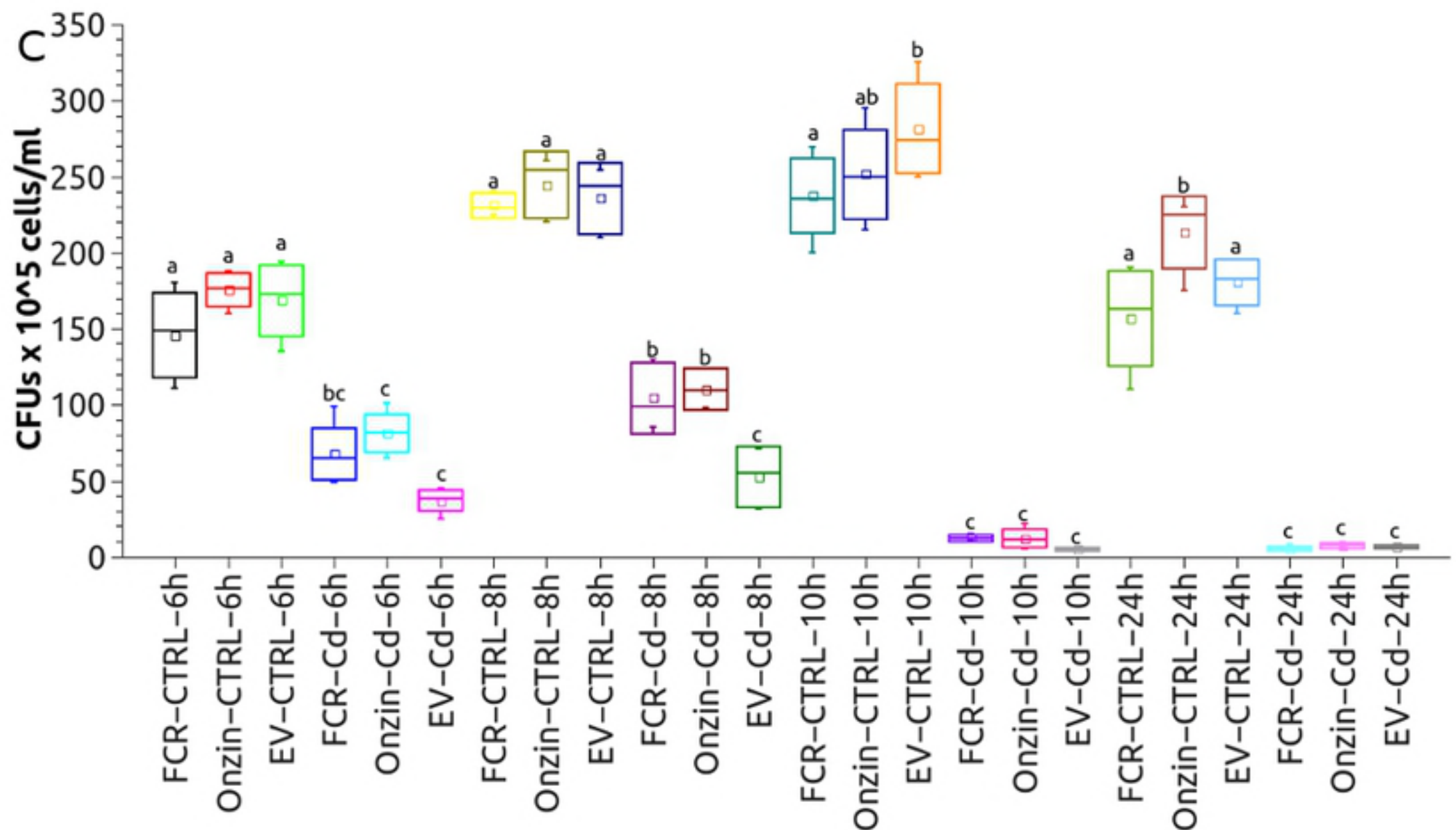
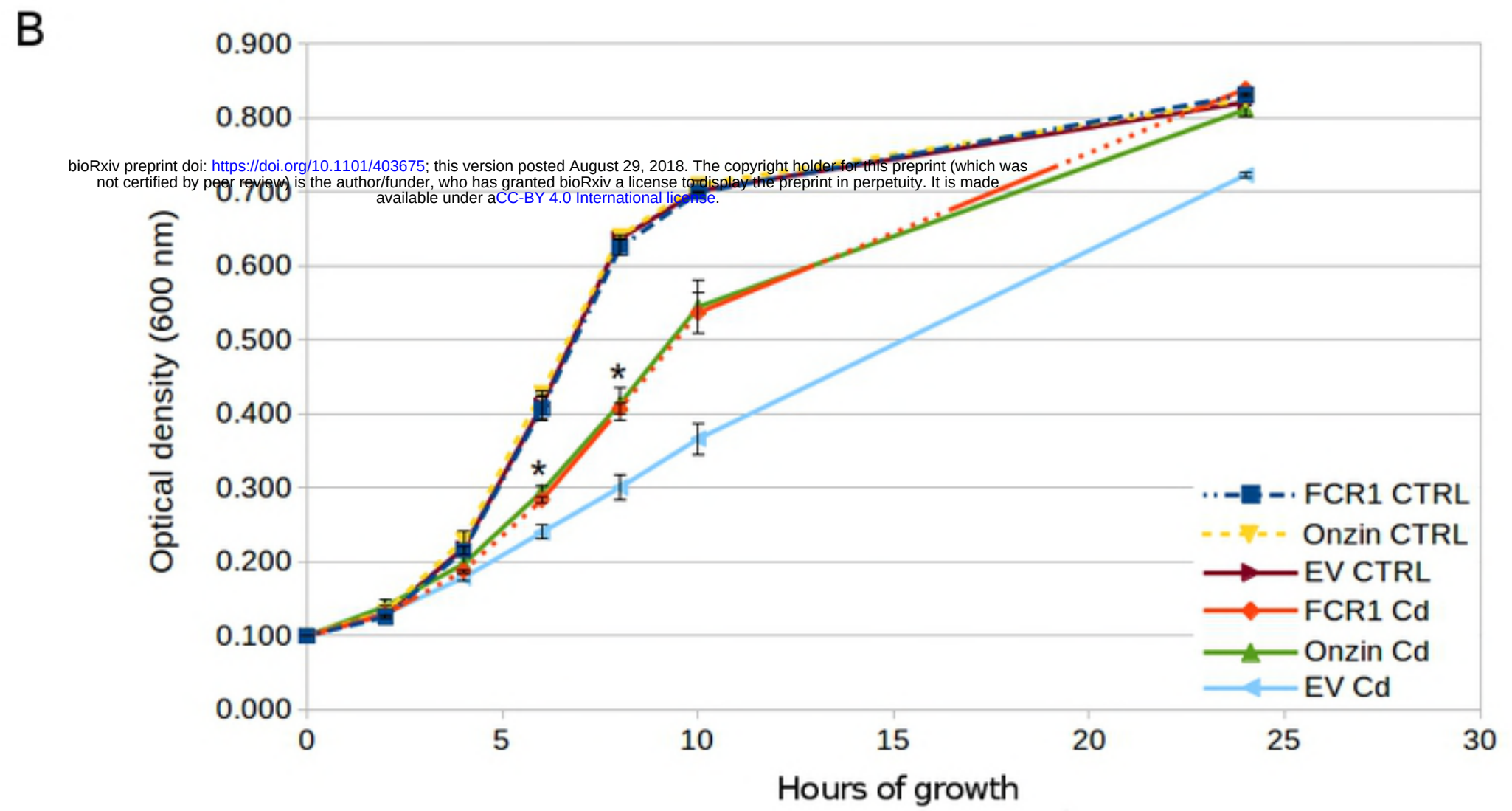
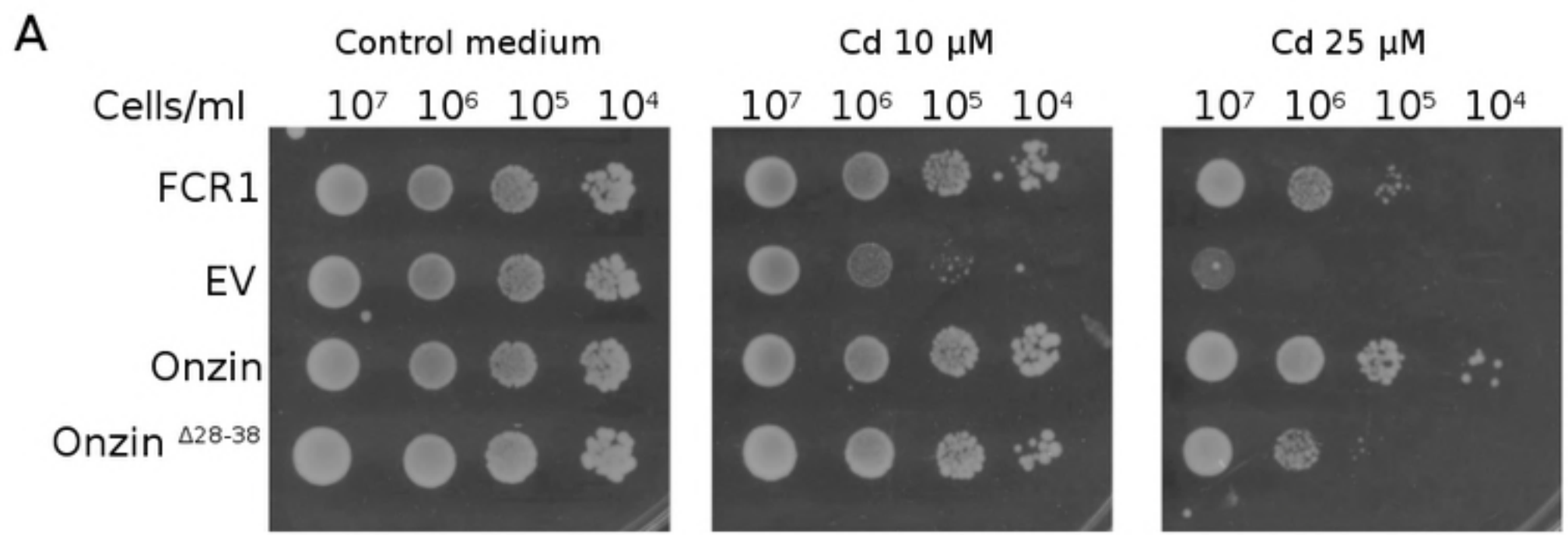
906

907 **S4 Data. Source data and detailed statistics for S3 Table.**

MmOnzin	---mAQAPt-----VivTQPgfvr--APQ-----NSNWQTsLCDCFS
HsOnzin	---MQAQAPV-----VvvTQPgvGPgpAPQ-----NSNWQTGmCDCFS
OmFCR1	mseIQeQAPIetkqaiteapasqaQPvaqPqqrPdglflaqhrvetgsneWkdGLlDCFSn

MmOnzin	C--GVCLCGTFCFtCL-----GCQvaADMNECCL-----CGTtvAM----R
HsOnzin	C--GVCLCGTFCFPCL-----GCQvaADMNECCL-----CGTSvAM----R
OmFCR1	apdnlCLkG-FCcPCfvvgktqarlrdpemkdyerfNtdCLmfvganyCGLSwlfpffrR

MmOnzin	TLYRTRYGIPGSICDDYmvTLfCPvCsvCQLKRDI nRRRAMnaF-----
HsOnzin	TLYRTRYGIPGSICDDYmaTLCCPhCTLCQiKRDI nRRRAMrTF-----
OmFCR1	TdiRTmYdIrGnvlGdcgsafCCLpCTLiQnekevi hRqtlpTtdkagyqavadmnagqq

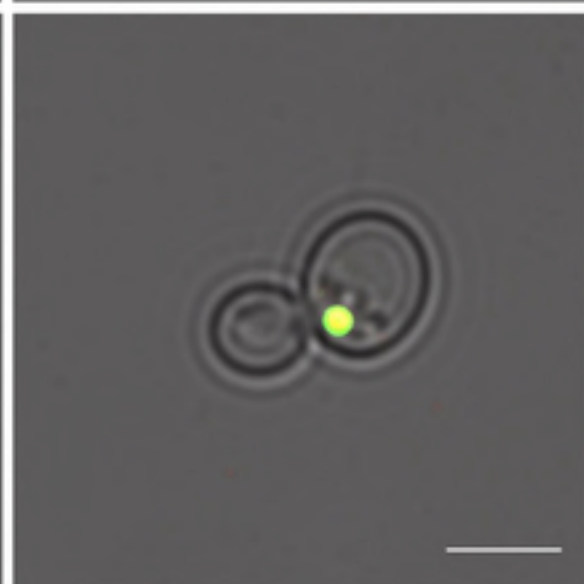
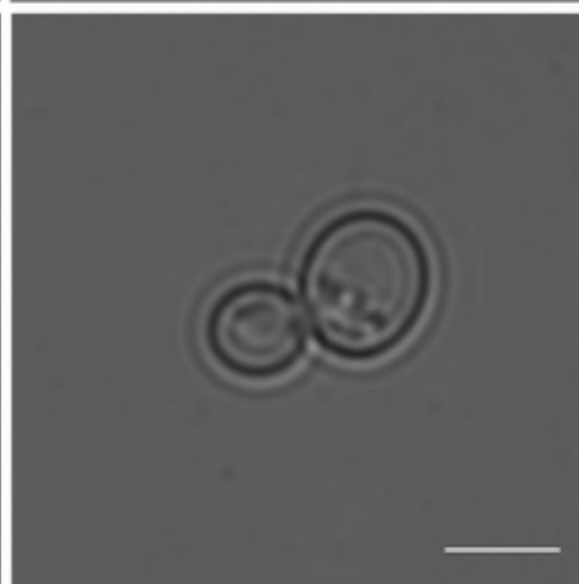
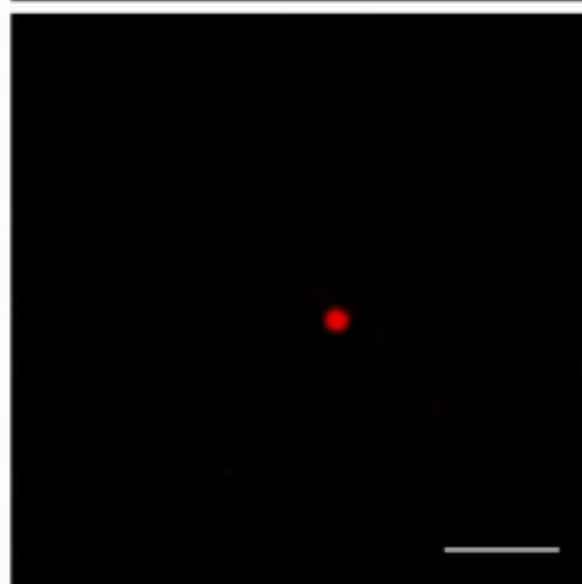
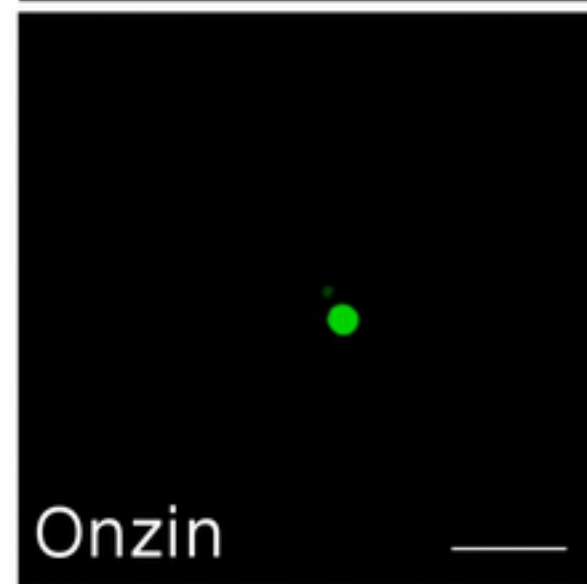
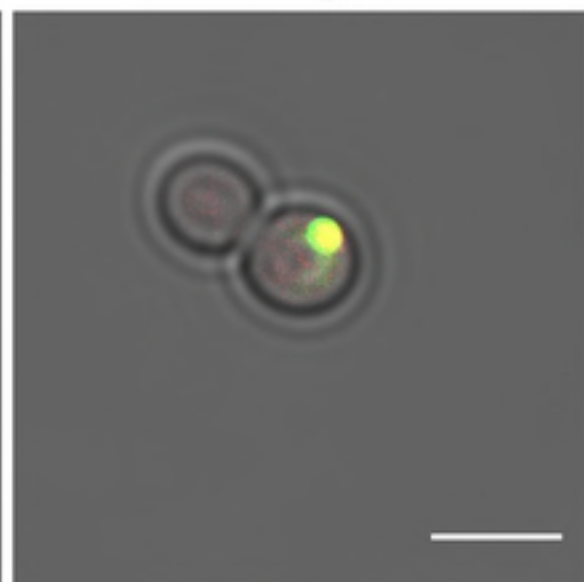
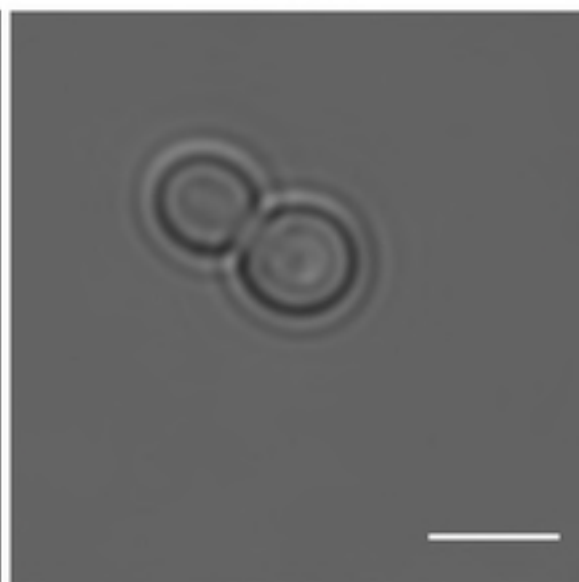
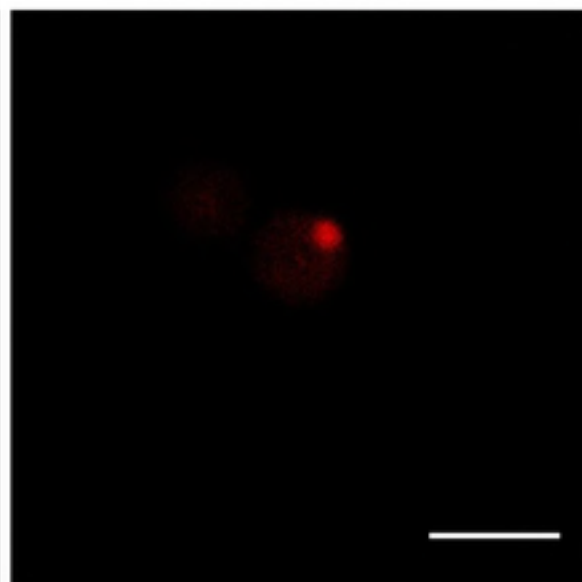
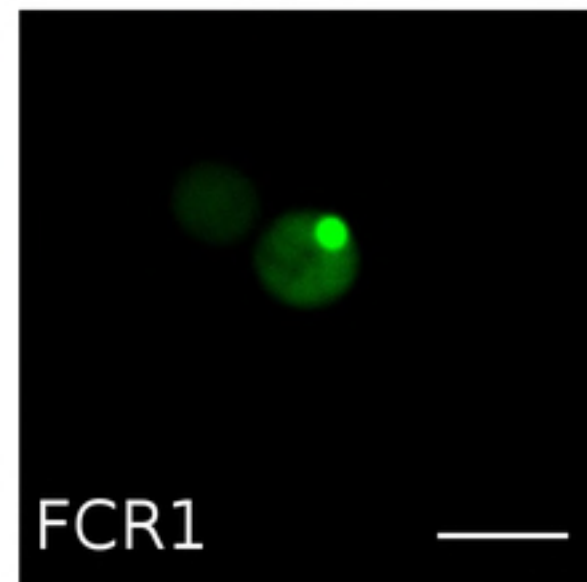


GFP

NLS-RFP

BF

Merge

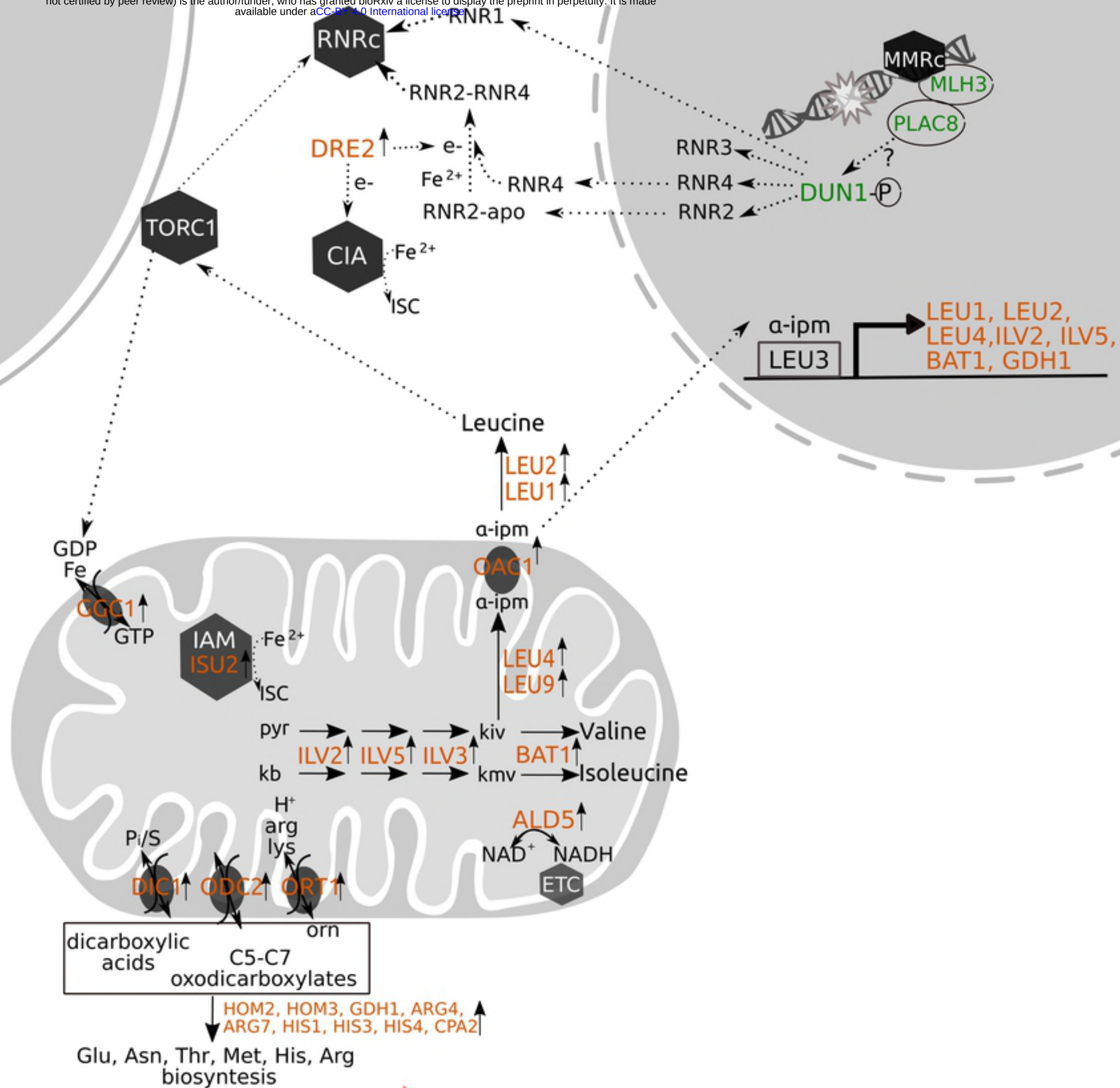


Vacuole

Nucleus

DNA replication and repair

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Control medium

Cd 10 μ MCell/ml 10^6 10^5 10^4 10^3 10^2 10^6 10^5 10^4 10^3 10^2 *dun1* EV*dun1* FCR1*dun1* Onzin

WT EV

WT FCR1

WT Onzin

