

Fig.S1 (Alkim *et al.*)

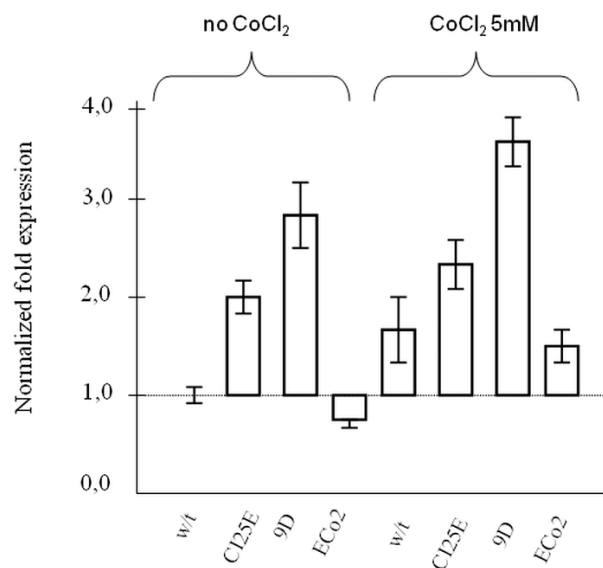


Figure S1: Validation by qRT-PCR of transcriptional increase of *COT1* in the evolved CI25E strains. Yeast cells were cultivated in SD medium in the absence up to 0.5-0.6 OD₆₀₀. Cobalt was added to part of the culture at 2 mM final concentration. After 1 h of incubation, samples were taken for RNA extraction. The extraction was performed using the SV Total RNA Isolation System (Promega). RT-PCR experiment was performed by using IQ™ SYBR® Green Supermix (*BIO-RAD*) kit. Use of reference genes and quantification of the transcript levels were done as described in ⁶².

Fig. S2 (Alkim *et al.*)

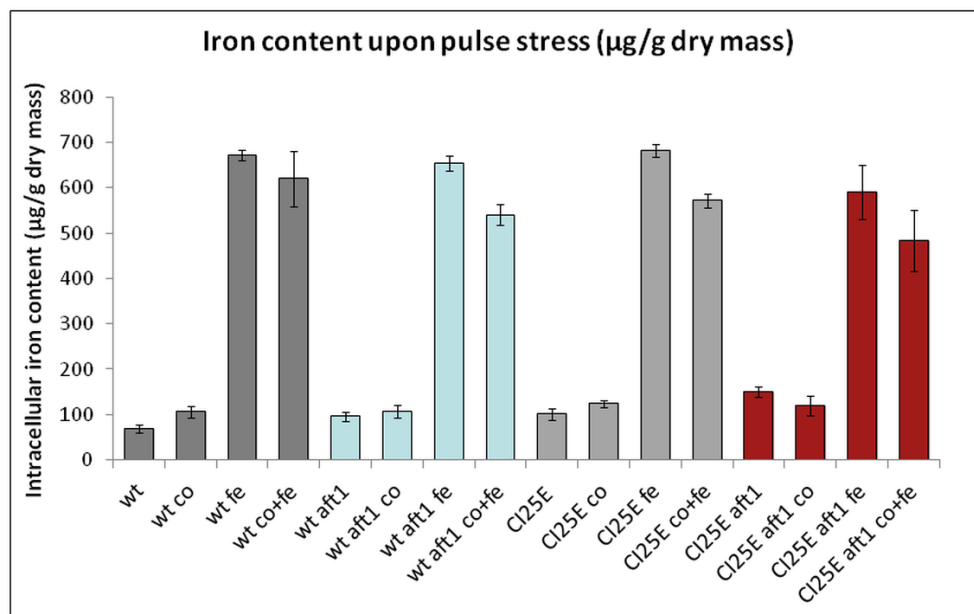


Figure S2: Intracellular levels of iron and cobalt ions in wild type CEN.PK 113.7 and evolved CI25E strain, bearing *AFT1* or deleted for this gene upon cobalt or iron stress.

Strains CEN.PK 113.7D *AFT1*, CEN.PK 113.7D *aft1* Δ , CI25E *AFT1* and CI25E *aft1* Δ were cultivated in SD medium to mid logarithmic phase ($OD_{600} = 1$ unit). At this time, the cultures were divided into 4 parts, to which $CoCl_2$ (2mM), $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (2mM) or both ions at 2 mM were added to each of them. After 90 min incubation, samples were taken for metal ions measurement as described in Material and Methods. The results shown are the mean \pm SEM of three independent experiments.

Table S1: List of oligonucleotides designed for gene deletion, gene sequencing, and RT-PCR analysis

name	Primer sequences for deletion	additional description
aft1::KANMX4_for	TCGGTGCATTGACAACCTC	
aft1::KANMX4_rev	AGCACTACTATTACACCAG	
aft1_verification_fi	CGCGAGATCCTAATAACCAAAC	
aft2::KANMX4_for	CCTCAGTTTACTTTATACAAAG	
aft2::KANMX4_rev	CGTGAACCATATTATAATCAACA	
aft2_verification_fi	TTAGAAATTTCAATAGCAAGTACT	
cot1::KANMX4_for	TATAGCAATTGCTGCGGAC	
cot1::KANMX4_rev	TGCAAGACTATTGCTATTATATTA	
cot1_verification_r	TTCCAGTAAACTCTTCAATT	
cot1::NATMX_for	catagctatagaagaagttacacaaagtcaggaagattgagtaaatCAGTATAGCGACCAGCAT	The sites for the NATMX marker are shown in upper case and homologous recombination regions in lower case.
cot1::NATMX_rev	taccgtataacgatttttaaaagtatttaattcttcacgcttttctgataaagtcctGACATGGAGGCCAC	The sites for the NATMX marker are shown in upper case and homologous recombination regions in lower case.
KANMX4_for	TGTTGATGCGCTGGCAGTG	
KANMX4_rev	CCATACAATCGATAGATTGT	
NATMX_for	GAGGCCATCGAGGCACTG	
NATMX_rev	TCGTACAGGGCGGTGTCC	
zrc1::KANMX4_for	TCTAATATGATTTCTTGGCATAG	
zrc1::KANMX4_rev	CACACCGAAGTGAGCTTAATT	
zrc1_verification_r	ACATTTGAAAGATCCATGAAAGT	
ura3::NATMX_for	tattaaccaactgcacagaacaaaaactgcaggaacgaagataaatcCAGTATAGCGACCAGCAT	The sites for the NATMX marker are shown in upper case and homologous recombination regions in lower case.
ura3::NATMX_rev	gctctaattgtgagtttagtatacatcattactataatacagtttGACATGGAGGCCAGAATAC	The sites for the NATMX marker are shown in upper case and homologous recombination regions in lower case.
Primer sequences for sequencing (AFT1, COT1, ZRC1)		
AFT1_seq_for	AATAACACTAGTGAGGGAAGTA	
AFT1_seq_rev	CCTGATCTTAGACCAGATATAT	
COT1_seq_for	TATAGCAATTGCTGCGGAC	
COT1_seq_rev	TGCAAGACTATTGCTATTATATTA	
ZRC1_seq_for	AAGGAAATGATATGAAAGTAGTTG	
ZRC1_seq_rev	AGATGAGGAAGGATTGTGGC	
MSN5_seq1_for	AGAGCAAGATTCATTGAACAG	
MSN5_seq1_rev	GTTCTTTTGGCACCTCTACC	
MSN5_seq2_for	AGGATTACTAGACCATGCAGTG	
MSN5_seq2_rev	TACTATAGGGACGGGTCAACA	
MSN5_seq3_for	ACTGGCCATTAACAGAGGTCA	
MSN5_seq3_rev	GTGGAACCTAAGACCTGCTGAC	
MSN5_seq4_for	TAGGTTGATTTCTTGCCTCGA	
MSN5_seq4_rev	GCAAATCAGAAAGACCAACAAC	
MSN5_seq5_for	TACCCAGACCTTGAAAGTGTC	
MSN5_seq5_rev	CCTAATGATGTGTCTACGGAG	
MSN5_seq6_for	ACATATGAAAGCGATGCAAACC	
MSN5_seq6_rev	TCATCCACCGTTGTTTTG	
MSN5_seq7_for	TGAAATGCTGCTTAACCTCAGT	
MSN5_seq7_rev	AGAAATACCTTGTTCAGACCCA	
PSE1_seq1_for	AGCCACTTTATCCTAAC	
PSE1_seq1_rev	ACCTAACTGGACCACTC	
PSE1_seq2_for	ACTGATGCAAGTGATAATGTCA	
PSE1_seq2_rev	TCATCCAAGAGTACAGTATG	
PSE1_seq3_for	CGTCAAGCTCTTGATCGTGT	
PSE1_seq3_rev	GAAATCAGCTTTGAGAGT	
PSE1_seq4_for	TATGGAATGTGCAACTCTG	
PSE1_seq4_rev	GCAATTGTCACCATGA	
PSE1_seq5_for	CGGAGGCTTAATGTGAGAA	
PSE1_seq5_rev	CTACGTTAGGAATGTTGGA	
PSE1_seq6_for	TCTTCAACAGAGAATGCC	
PSE1_seq6_rev	GATACCATACCTTATCGAGT	
Primer sequences for quantitative RT-PCR		
ACT1_QPCR_for	ATTATATGTTTAGAGGTTGCTGCTTTGG	
ACT1_QPCR_rev	AATTCGTTGTAGAAGGTATGATGCC	
AFT1_QPCR_for	ATGCATCTAAAAGGCCATGC	
AFT1_QPCR_rev	GGCAGTGGAAGATTTTCATT	
COT1_QPCR_for	GCATGGTGTGTTCTTCACG	
COT1_QPCR_rev	GGAAGCCTTGACAGATAGAG	

Table S2a: Complete list of upregulated genes in CI25E

	Systematic name	Gene name	logFC	fold change	p.value
1	YLR134w	PDC5	5,517619	45,8109009	2,99E-12
2	YMR058w	FET3	3,680726	18,3318197	3,54E-09
3	YLR136c	TIS11/CTH2	4,196278	18,3318197	3,54E-09
4	YHL040c	ARN1	3,336741	12,8235678	3,54E-09
5	YOR383c	FIT3	2,952205	10,1032027	1,79E-08
6	YNL237w	YTP1	2,873258	7,73930842	3,54E-09
7	YEL065w	SIT1	2,821915	7,32717967	2,80E-08
8	YLR214w	FRE1	2,609919	7,07100145	1,94E-08
9	YDR270w	CCC2	2,483814	6,10469403	7,40E-09
10	YOL158c	ENB1	2,419026	5,59374205	3,54E-09
11	YKL163w	PIR3	2,22548	5,34809808	2,00E-08
12	YER145c	FTR1	2,167321	4,67666385	9,14E-09
13	YNL160w	YGP1	2,14057	4,49188533	1,79E-08
14	YKL220c	FRE2	2,131905	4,40936275	1,62E-07
15	YJL079c	PRY1	2,106897	4,38295728	9,14E-09
16	YHL035c	VMR1	2,066273	4,30763871	9,14E-09
17	YPR124w	CTR1	1,838017	4,18803311	2,80E-07
18	YCL025c	AGP1	1,754655	3,57518287	8,38E-08
19	YDR059c	UBC5	1,632038	3,37445575	1,50E-06
20	YLR121c	YPS3	1,628915	3,09950633	4,16E-06
21	YGR259c	YGR259c	1,551257	3,09280334	6,32E-06
22	YOR382w	FIT2	1,53846	2,93072303	2,01E-05
23	YLL051c	FRE6	1,508217	2,9048426	1,64E-05
24	YLR205c	HMX1	1,488217	2,84458175	4,09E-07
25	YDR046c	BAP3	1,487493	2,80541952	5,26E-06
26	YGR110w	CLD1	1,394593	2,80401315	1,21E-06
27	YIL169c	YIL169c	1,393307	2,62914386	2,88E-05
28	YPL274w	SAM3	1,386136	2,62680066	1,87E-06
29	YOL155c	HPF1	1,369926	2,6137773	8,75E-06
30	YLR194c	YLR194c	1,344165	2,58457329	6,57E-05
31	YOR381w	FRE3	1,333883	2,53883167	1,75E-06
32	YFL057c	AAD16	1,313539	2,52080272	1,21E-06
33	YFL056c	AAD6	1,308496	2,48550443	1,93E-06
34	YFL030w	AGX1	1,265065	2,47683178	2,31E-06
35	YOR072w	YOR072w	1,257712	2,40337972	2,04E-05
36	YFL041w	FET5	1,22135	2,39116175	4,42E-06
37	YGR189c	CRH1	1,218322	2,33164856	8,26E-05
38	YPL052w	OAZ1	1,216537	2,32675855	0,00017752
39	YJL153c	INO1	1,189061	2,32388186	5,93E-06
40	YBR295w	PCA1	1,185105	2,28004311	7,87E-05
41	YHR138c	YHR138c	1,182035	2,27379912	3,35E-06
42	YMR056c	AAC1	1,180134	2,26896679	1,50E-05
43	YGR043c	NQM1	1,157919	2,26597897	8,90E-06

44	YLR327c	TMA10	1,11867	2,23135339	0,0114313
45	YGL121c	GPG1	1,110364	2,17146739	0,00052541
46	YAR068w	YAR068w	1,098757	2,1590009	0,0003748
47	YBL043w	ECM13	1,095811	2,14170139	1,49E-05
48	YBR071w	YBR071w	1,088984	2,13733267	7,46E-06
49	YHR048w	YHK8	1,084842	2,1272422	0,00021259
50	YBL048w	YBL048w	1,067366	2,12114316	0,00014907
51	YDL169c	UGX2	1,04943	2,09560393	4,58E-05
52	YLL058w	YLL058w	1,037509	2,06971209	2,96E-05
53	YHR214w-a	YHR214w-a	1,033619	2,05268013	0,0008639
54	YNL202w	SPS19	1,029691	2,04715331	4,34E-05
55	YLR030w	YLR030w	1,021225	2,04158736	5,51E-05
56	YER175c	TMT1	1,02104	2,02964091	7,60E-05
57	YIL101c	XBP1	1,006256	2,01229912	0,00206695
58	YKR013w	PRY2	0,994925	2,00869121	7,73E-05
59	YLL057c	JLP1	0,993123	1,99297712	0,0004855
60	YLR047c	FRE8	0,982251	1,9904891	0,00024304
61	YGL071W	AFT1	0,977694	1,97554566	7,49E-05
62	YLR034c	SMF3	0,976111	1,96931556	2,81E-05
63	YGR065c	VHT1	0,97513	1,9671552	0,00034427
64	YGR279c	SCW4	0,959286	1,9658178	6,07E-05
65	YDR264c	AKR1	0,956932	1,94434674	5,02E-05
66	YMR002w	MIC17	0,944878	1,94117705	2,47E-05
67	YGR055w	MIUP1	0,942225	1,92502571	0,00095054
68	YLL056c	YLL056c	0,93279	1,92148991	0,00030735
69	YOL153c	YOL153c	0,931328	1,90896466	9,35E-05
70	YNL289w	PCL1	0,930473	1,90702995	0,00034897
71	YMR034c	YMR034c	0,930324	1,9059012	0,00016953
72	YPR151c	SUE1	0,919206	1,90570439	0,00010033
73	YPL156c	PRM4	0,909514	1,89107472	0,00020306
74	YBR207w	FTH1	0,909094	1,87841306	0,00034324
75	YGL104c	VPS73	0,901518	1,87786529	6,86E-05
76	YDR055w	PST1	0,897921	1,86802994	4,08E-05
77	YBR046c	ZTA1	0,897885	1,86337886	9,52E-05
78	YOR385w	YOR385w	0,894488	1,86333242	6,58E-05
79	YGR079w	YGR079w	0,889465	1,85894955	0,00157442
80	YPL111w	CAR1	0,883549	1,85248929	0,00033493
81	YOR338w	YOR338w	0,869806	1,84490766	0,0005375
82	YCL026C-B	HBN1	0,856596	1,82741726	9,74E-05
83	YLR414c	PUN1	0,856404	1,81076134	9,74E-05
84	YJL185c	YJL185c	0,855881	1,81051944	0,00039283
85	YDL021w	GPM2	0,850822	1,80986363	0,0002146
86	YIL042c	PKP1	0,845466	1,80352837	6,21E-05
87	YPR001w	CIT3	0,845388	1,79684549	0,00076605
88	YER064c	YER064c	0,842639	1,79674822	0,00101164
89	YAL062w	GDH3	0,840768	1,79332717	0,00033331

90	YBL049w	MOH1	0,838385	1,79100382	0,00087947
91	YHL026c	YHL026c	0,836229	1,78804726	0,00018902
92	YML043c	RRN11	0,825606	1,78537688	0,00584027
93	YLR031w	YLR031w	0,825301	1,77227964	0,0003748
94	YPL277c	YPL277c	0,819092	1,7719045	0,00022459
95	YER039c	HVG1	0,816655	1,76429529	0,00015798
96	YKR091w	SRL3	0,816379	1,76131703	0,00034883
97	YLR234w	TOP3	0,813532	1,76098046	0,00082547
98	YGL156w	AMS1	0,808088	1,75750845	0,00088975
99	YEL038w	UTR4	0,807983	1,75088926	0,00012726
100	YJL159w	HSP150	0,800409	1,75076176	0,000555
101	YPL163c	SVS1	0,795167	1,74159493	0,00017086
102	YMR191w	SPG5	0,793444	1,73527814	0,00019758
103	YKR049c	FMP46	0,790029	1,73320697	0,00149499
104	YLR324w	PEX30	0,785604	1,72910967	0,00066002
105	YGR213c	RTA1	0,783741	1,72381409	0,00204616
106	YBR294w	SUL1	0,775379	1,72158926	0,00326117
107	YOR185c	GSP2	0,773771	1,71164017	0,00019498
108	YLR213c	CRR1	0,769931	1,70973237	0,00350762
109	YEL060c	PRB1	0,763791	1,7051886	0,00096814
110	YFL055w	AGP3	0,762462	1,69794706	0,00129814
111	YPR030w	CSR2	0,760644	1,69638308	0,00264597
112	YIR038c	GTT1	0,753336	1,69424681	0,0001652
113	YDR379c-a	YDR379c-a	0,753223	1,68568563	0,00048135
114	YAL053w	FLC2	0,746565	1,68555394	0,00048135
115	YER004w	FMP52	0,745098	1,67779308	0,00016607
116	YKR041w	YKR041w	0,736618	1,6760877	0,01906715
117	YOR208w	PTP2	0,734098	1,66626573	0,00086449
118	YLR046c	YLR046c	0,730795	1,66335765	0,00053574
119	YEL020c	YEL020c	0,730781	1,65955307	0,00093523
120	YPL278c	YPL278c	0,728435	1,65953691	0,00044212
121	YHL024w	RIM4	0,723426	1,65684046	0,00669229
122	YLL039c	UBI4	0,723327	1,65109781	0,00049159
123	YBR026C	MRF1'	0,721219	1,65098538	0,00048135
124	YNL065w	AQR1	0,718366	1,64857455	0,00354952
125	YHR137w	ARO9	0,714694	1,64531804	0,00255186
126	YMR145c	NDE1	0,71039	1,64113465	0,0018515
127	YNL305c	YNL305c	0,708684	1,63624592	0,0009724
128	YKR061w	KTR2	0,704456	1,63431311	0,0044098
129	YBR182c	SMP1	0,703542	1,62953022	0,00085073
130	YLR237w	THI7	0,698405	1,62849803	0,00074664
131	YLL060c	GTT2	0,698059	1,62271021	0,00051073
132	YLR063w	YLR063w	0,697902	1,62232107	0,00953146
133	YMR148w	OSW5	0,69586	1,62214365	0,00075443
134	YJL151c	SNA3	0,694427	1,61985015	0,00039995
135	YPL104w	MSD1	0,691723	1,61824197	0,00076605

136	YJL149w	DAS1	0,687597	1,61521166	0,00106758
137	YJL116c	NCA3	0,685625	1,61059895	0,00494412
138	YPL149W	ATG5	0,681063	1,60839874	0,00099962
139	YHL010c	ETP1	0,680876	1,60332114	0,00469771
140	YHL016c	DUR3	0,678851	1,60311311	0,00558467
141	YER062c	HOR2	0,678779	1,60086427	0,0014873
142	YDL130w-a	STF1	0,678607	1,60078461	0,00230962
143	YDR525w-a	SNA2	0,676428	1,60059368	0,00420778
144	YER095w	RAD51	0,672315	1,59817831	0,00051073
145	YBR116c	YBR116c	0,664785	1,59362774	0,00121921
146	YGL209w	MIG2	0,663714	1,58533201	0,00171177
147	YDR476c	YDR476c	0,662118	1,58415551	0,00066002
148	YJL117w	PHO86	0,656304	1,58240353	0,00048135
149	YJR019c	TES1	0,652618	1,57604006	0,00090547
150	YPL107w	YPL107w	0,652607	1,57201843	0,00197372
151	YOL119c	MCH4	0,649544	1,57200647	0,00057046
152	YML038c	YMD8	0,648869	1,56867217	0,00124059
153	YHR209w	CRG1	0,646456	1,56793841	0,00212902
154	YBR005w	RCR1	0,645861	1,5653186	0,00092586
155	YNL282w	POP3	0,645806	1,56467287	0,0130576
156	YKR046c	PET10	0,643772	1,56461304	0,00051292
157	YMR035w	IMP2	0,642661	1,56240836	0,00479393
158	YOL083w	YOL083w	0,642002	1,5612065	0,00658531
159	YOR389w	YOR389w	0,638607	1,56049346	0,00138527
160	YOR289w	YOR289w	0,637931	1,55682574	0,00289643
161	YGL096w	TOS8	0,637538	1,5560962	0,01664795
162	YMR305c	SCW10	0,63688	1,55567208	0,00384144
163	YNL083w	SAL1	0,635073	1,55496264	0,00157442
164	YNL208w	YNL208w	0,633328	1,55301673	0,00575423
165	YPR193c	HPA2	0,632702	1,55113883	0,00978502
166	YLR142w	PUT1	0,630827	1,55046596	0,00980252
167	YMR280c	CAT8	0,629989	1,54845187	0,00263016
168	YNL300w	TOS6	0,628935	1,54755353	0,00111371
169	YMR238w	DFG5	0,624456	1,54642271	0,0026006
170	YJL082w	IML2	0,623476	1,54162978	0,00085073
171	YER142c	MAG1	0,622209	1,54058223	0,00194469
172	YBR269c	FMP21	0,622123	1,5392301	0,00098313
173	YMR304c-a	YMR304c-a	0,619956	1,53913798	0,00274065
174	YLR126c	YLR126c	0,619835	1,53682787	0,00129637
175	YPR157w	YPR157w	0,616292	1,53669949	0,04503465
176	YGR153w	YGR153w	0,614781	1,53293068	0,00255186
177	YGR149w	YGR149w	0,614484	1,53132576	0,00089199
178	YOL122c	SMF1	0,613192	1,53100999	0,00194993
179	YGL146c	RRT6	0,612997	1,52963993	0,00358661
180	YLR174w	IDP2	0,61207	1,52943331	0,00899779
181	YOL048c	RRT8	0,610067	1,52845036	0,00504818

182	YCR060w	TAH1	0,605413	1,52633024	0,0028882
183	YOR359w	VTS1	0,605112	1,52141392	0,02181374
184	YLR120c	YPS1	0,604464	1,5210968	0,00149046
185	YDR077w	SED1	0,603914	1,52041393	0,00211517
186	YDR492w	IZH1	0,602805	1,51983415	0,00240174
187	YDL070w	BDF2	0,602511	1,51866609	0,01175348
188	YLL059c	YLL059c	0,602403	1,5183571	0,00819349
189	YDL144c	YDL144c	0,597185	1,51824355	0,00099962
190	YPL134c	ODC1	0,595056	1,5127616	0,00255186
191	YGL029w	CGR1	0,594227	1,51053161	0,0242477
192	YEL059w	YEL059w	0,592057	1,50966385	0,00933016
193	YIR018w	YAP5	0,591646	1,50739408	0,00202916
194	YHR057c	CPR2	0,590634	1,50696554	0,00110842
195	YDR265w	PEX10	0,589364	1,50590879	0,00205087
196	YKL175w	ZRT3	0,588737	1,50458301	0,00494412
197	YIL150C	MCM10	0,587823	1,50392963	0,01012526
198	YBR267w	REI1	0,587524	1,5029769	0,01649833
199	YGL047w	ALG13	0,58646	1,50266565	0,00118708
200	YKR076w	ECM4	0,584285	1,50155773	0,00163667

Table S2b: List of downregulated genes in the cobalt resistant CI25E strain

	Systematic	gene	logFC	fold change	p.value
1	YGR088w	CTT1	-1,948547728	0,227130717	0,00012751
2	YIL014c-a	YIL014c-a	2,13E-05	0,259076896	0,99987926
3	YIL006w	YIA6	0,00030776	0,279384201	0,99896558
4	YJR025c	BNA1	-1,839677658	0,319215411	3,56E-07
5	YIL015w	BAR1	-1,647397791	0,326723074	2,53E-07
6	YIR030c	DCG1	-1,61385975	0,334831044	2,97E-07
7	YER103w	SSA4	-1,578494799	0,347274829	0,01031094
8	YCL021w-a	YCL021w-a	-1,525850251	0,347702164	1,39E-05
9	YAL068c	PAU8	-1,524076049	0,353884265	4,09E-07
10	YGL261c	PAU11	-1,498650478	0,358422466	1,21E-06
11	YBL107w-a	YBL107w-a	-1,480267027	0,364688389	1,51E-06
12	YMR105c	PGM2	-1,455263827	0,37730689	0,00226877
13	YGR052w	FMP48	-1,406189651	0,38201444	0,00019498
14	YLR231c	BNA5	-1,388300921	0,385671375	1,18E-06
15	YDR542w	PAU10	-1,374556023	0,386319641	1,50E-06
16	YGR142w	BTN2	-1,372133068	0,38686054	0,00662688
17	YGR294w	PAU12	-1,370114515	0,390120875	1,21E-06
18	YBR072w	HSP26	-1,358006896	0,396022393	0,01278431
19	YOR032c	HMS1	-1,336346085	0,399140274	2,01E-06
20	YJL223c	PAU1	-1,325032238	0,402643269	2,74E-06
21	YAL061w	BDH2	-1,312425878	0,404889893	0,00234874
22	YNR075w	COS10	-1,304398464	0,413738937	7,46E-06
23	YNR068c	YNR068c	-1,27320736	0,41949924	4,11E-05
24	YFR015c	GSY1	-1,253259899	0,420033275	0,0004479
25	YOR071c	NRT1	-1,251424472	0,423805405	7,68E-06
26	YML054c	CYB2	-1,238526108	0,428461148	0,00075443
27	YBL108c-a	PAU9	-1,222763706	0,430424356	3,17E-06
28	YOR161c	PNS1	-1,216168379	0,433564891	0,00442605
29	YLR304c	ACO1	-1,205680158	0,436809084	1,11E-05
30	YBR083w	TEC1	-1,194925235	0,441723974	5,93E-06
31	YHL046c	PAU13	-1,17878296	0,443242998	5,20E-06
32	YBR301W	DAN3	-1,173830253	0,443769517	9,65E-06
33	YFL026w	STE2	-1,172117524	0,444894927	5,62E-06
34	YDR516c	EMI2	-1,168463446	0,445780543	0,00034139
35	YER138w-a	YER138w-a	-1,165594445	0,445934363	0,00011375
36	YJR078w	BNA2	-1,16509672	0,448244289	0,00021323
37	YIL176c	PAU14	-1,157642892	0,448795234	2,58E-05
38	YGR248w	SOL4	-1,155870741	0,449681368	0,00919803
39	YNR014w	YNR014w	-1,153024985	0,456656175	0,00640747
40	YKL209c	STE6	-1,130819755	0,461266864	1,65E-05
41	YPR160w	GPH1	-1,116326439	0,47218523	0,01236419
42	YBL098w	BNA4	-1,082575181	0,480468853	2,96E-05
43	YIL119c	RPI1	-1,057485184	0,482056819	4,58E-05
44	YEL049w	PAU2	-1,052724892	0,483815984	2,06E-05

45	YLR461w	PAU4	-1,047469663	0,493725774	4,99E-05
46	YDR342c	HXT7	-1,018218136	0,496292713	0,01261195
47	YJL088w	ARG3	-1,010736822	0,496743197	0,0001652
48	YNR076w	PAU6	-1,009427885	0,499299933	1,11E-05
49	YLL064c	PAU18	-1,002021383	0,505146286	3,63E-05
50	YHR055c	CUP1-2	-0,985226856	0,505581256	0,00048135
51	YHR216w	IMD2	-0,983985117	0,508447996	2,36E-05
52	YHR053c	CUP1-1	-0,975827871	0,512185678	3,63E-05

Table S3: Main transcription factors that were identified from enriched functional categories listed in Table 2 & 3 from transcriptome profile of the cobalt resistant CI25E strain*

Main Functional category	Transcription factor	Description of the biological function	% relative to total genes in the list regulated by this TF	% relative to the total number of genes regulated by this TF
Metal ions (Cu ⁺ , Fe ²⁺ , Fe ³⁺ , etc.) transport, and homeostasis	Aft1	Transcription factor involved in iron utilization and homeostasis; binds the consensus site PyPuCACCCPu and activates the expression of target genes in response to changes in iron availability	76	1.8
	Aft2	Iron-regulated transcriptional activator; activates genes involved in intracellular iron use and required for iron homeostasis and resistance to oxidative stress; similar to Aft1p	64	6.8
	Put3	Transcriptional activator of proline utilization genes, constitutively binds PUT1 and PUT2 promoter sequences; has a Zn(2)-Cys(6) binuclear cluster domain	68	10.3
Transmembrane transport (for C-compounds, carbohydrates, amino acids and amino acids derivatives)	Ste2	Transcription factor that is activated by a MAP kinase signaling cascade, activates genes involved in mating or pseudohyphal/invasive growth pathways; cooperates with Tec1p transcription factor to regulate genes specific for invasive growth	56	0.4
	Yap1	Basic leucine zipper (bZIP) transcription factor required for oxidative stress tolerance; activated by H ₂ O ₂ through the multistep formation of disulfide bonds and transit from the cytoplasm to the nucleus; mediates resistance to cadmium	50	0.5
	Msn2	Transcriptional activator activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress response elements of responsive genes	50	0.7
Cell wall proteins, cell wall organization	Ste12	Transcription factor that is activated by a MAP kinase signaling cascade, activates genes involved in mating or pseudohyphal/invasive growth pathways; cooperates with Tec1p transcription factor to regulate genes specific for invasive growth	94	0.4
	Yap1	Basic leucine zipper (bZIP) transcription factor required for oxidative stress tolerance; activated by H ₂ O ₂ through the multistep formation of disulfide bonds and transit from the cytoplasm to the nucleus; mediates resistance to cadmium	71	0.5
Metabolic processes, with emphasis on carbon and amino acid metabolism	Yap1	Basic leucine zipper (bZIP) transcription factor required for oxidative stress tolerance; activated by H ₂ O ₂ through the multistep formation of disulfide bonds and transit from the cytoplasm to the nucleus; mediates resistance to cadmium	71.4	0.5
PAU family and other stress genes	Upc2	Sterol regulatory element binding protein, induces transcription of sterol transport and biosynthetic genes; involved in the anaerobic induction of DAN/TIR mannoproteins and seripauperins; binucleate zinc cluster protein	82	7.1
	Aft1	Transcription factor involved in iron utilization and homeostasis; binds the consensus site PyPuCACCCPu and activates the expression of target genes in response to changes in iron availability	76	1.8
Biosynthesis of vitamins, cofactors (NAD ⁺) and prosthetic group	Sum1	Transcriptional repressor required for mitotic repression of middle sporulation-specific genes; involved in telomere maintenance, regulated by the pachytene checkpoint	83	3.1
	Leu3	Zinc-finger transcription factor that regulates genes involved in branched chain amino acid biosynthesis and ammonia assimilation; positively regulated by alpha-isopropylmalate, an intermediate in leucine biosynthesis	83	1

*Yeasttract (www.yeasttract.com) has been used to search for TF associated to the functional categories

Table S4: List of the *AFT1*-dependent genes activation in the cobalt evolved strain CI25E compared to the documented *AFT1*-dependent activated genes taken from ³¹ and ⁴⁷ and compared with other conditions previously published.

From de Freitas <i>et al.</i> Figure 2 and Table 1 in JBC (2004)		From Philpott & Protchenko Table 1 in Euk.Cell (2008)		Alkim <i>et al.</i>	Li <i>et al.</i> , 2004	Stadler and Schweyen, 2002				de Freitas <i>et al.</i> , 2004	Hausmann <i>et al.</i> , 2008			Puig <i>et al.</i> , 2005						Veatch <i>et al.</i> , 2009						Ojeda <i>et al.</i> , 2006	Foury <i>et al.</i> , 2001	Shakoury-Elizeh <i>et al.</i> , 2004		
systematic name	Gene name	systematic name	Gene name	evolved strain	mrs3Δmrs4Δ	30 min; 100 μM CoCL2	30 min: 2 mMCoCl2	90 min: 2 mM CoCl2	90 min, 80 μM BPS	mac1Δ	atm1Δ	yah1Δ	wt	cth2Δ	3h	11h	19h	27h	ATP1-111	NAR1	grx3Δgrx4Δ 0,2 mM FeCl3	yfh1Δ	AFT1 ^{UP} vs. W/T	AFT1 ^{UP} vs. aft1 Δ	20 μM Fe vs. 100 μM Fe iron-poor vs. iron-sufficient					
Copper and iron homeostasis/Homeostasis to metal ions		Uptake of iron at the cell surface																												
YDR534C	<i>FIT1</i>	YDR534C	<i>FIT1</i>				UP	UP		UP	UP	UP	UP	UP	UP	UP	UP	UP	DOWN		UP	UP	UP	UP						
YOR382W	<i>FIT2</i>	YOR382W	<i>FIT2</i>	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	DOWN	UP	UP	UP	UP	UP					
YOR383C	<i>FIT3</i>	YOR383C	<i>FIT3</i>	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	DOWN	UP	UP	UP	UP	UP					
YLR214W	<i>FRE1</i>	YLR214W	<i>FRE1</i>	UP	UP					DOWN	UP	UP	UP		UP	UP	UP	UP				UP	UP	UP						
YKL220C	<i>FRE2</i>	YKL220C	<i>FRE2</i>	UP						UP	UP	UP										UP	UP	UP	UP					
YOR381W	<i>FRE3</i>	YOR381W	<i>FRE3</i>	UP	UP					UP	UP	UP	UP			UP	UP	UP	UP	DOWN		UP	DOWN	UP	UP					
YNR060W	<i>FRE4</i>	YNR060W	<i>FRE4</i>							DOWN													UP	UP	DOWN					
YOR384W	<i>FRE5</i>	YOR384W	<i>FRE5</i>							UP	UP	UP										UP	UP	UP						
YMR058W	<i>FET3</i>	YMR058W	<i>FET3</i>	UP	UP	UP	UP	UP		UP	UP	UP	UP		UP	UP	UP	UP	UP		UP	UP	UP	UP	UP					
YER145C	<i>FTR1</i>	YER145C	<i>FTR1</i>	UP	UP		UP	UP		UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN		UP	UP	UP	UP					
YNL259C	<i>ATX1</i>	YNL259C	<i>ATX1</i>							UP	UP				UP	UP	UP	UP	UP			UP	UP	UP	UP					
YDR270W	<i>CCC2</i>	YDR270W	<i>CCC2</i>	UP	UP					UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN		UP	UP	UP						
YHL040C	<i>ARN1</i>	YHL040C	<i>ARN1</i>	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP		UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP					
YHL047C	<i>ARN2/TAF1</i>	YHL047C	<i>ARN2/TAF1</i>		UP	UP	UP	UP		UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN		UP	UP	UP	UP					
YEL065W	<i>ARN3/SIT1</i>	YEL065W	<i>ARN3/SIT1</i>	UP	UP					UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN	UP	UP	DOWN	UP	UP					
YOL158C	<i>ARN4/ENB1</i>	YOL158C	<i>ARN4/ENB1</i>	UP	UP	UP	UP	UP	UP	UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN	UP	UP	UP	UP	UP					
YOR264c	<i>AKR1</i>			UP				UP		UP	UP	UP										UP	UP	UP	UP					
YGL047c	<i>AFT1</i>			UP						UP																				
YPR124w	<i>CTR1</i>			UP																										
YHR175w	<i>CTR2</i>																													
YLR411w	<i>CTR3</i>																													
YMR319c	<i>FET4</i>			DOWN						DOWN	UP	UP													UP					
YOR176w	<i>HEM15</i>			DOWN						DOWN	DOWN	DOWN	UP		DOWN	DOWN	DOWN	DOWN	DOWN	DOWN					DOWN					
YMR021c	<i>MAC1</i>																													
YBR295w	<i>PCA1</i>			UP						UP																				
YLR221w	<i>CCC1</i>			DOWN						DOWN	DOWN	DOWN	UP																	
YDR423c	<i>CAD1/YAP2</i>									UP			UP								UP									
YKL175w	<i>ZRT3</i>			UP						UP																				
YOL152w	<i>FRE7</i>			UP						UP																				
		Efflux of iron from vacuole to cytosol																												
		YLL051C	<i>FRE6</i>	UP									UP		UP	UP	UP	UP	UP	UP			UP	UP	UP					
		YLR034C	<i>SMF3</i>	UP							UP												UP	UP	UP					
YFL041W	<i>FET5</i>	YFL041W	<i>FET5</i>	UP					UP	UP	UP	UP	UP		UP	UP	UP	UP	UP	DOWN		UP	UP	UP						
		YBR207W	<i>FTH1</i>	UP						UP			UP			UP	UP	UP	UP	DOWN		UP	UP	UP	UP					
		Other transporters																												
		YGR065C	<i>VHT1</i>	UP							UP												UP	UP	UP					
YOR316C	<i>COT1</i>	YOR316C	<i>COT1</i>	UP	UP		UP			UP		UP										UP	UP	UP	UP					
YKR052C	<i>MRS4</i>	YKR052C	<i>MRS4</i>							UP												UP	UP	UP	UP					
		Metabolic adaptation to low iron																												
		YLR205C	<i>HMX1</i>	UP				UP		UP	UP	UP	UP	UP		DOWN			UP			UP	UP	UP	UP					
YLR136C	<i>CTH2/TIS11</i>	YLR136C	<i>CTH2/TIS11</i>	UP		UP	UP			UP	UP	UP	UP	UP	UP	UP	UP	UP	UP	DOWN	UP	UP	UP	UP	UP					