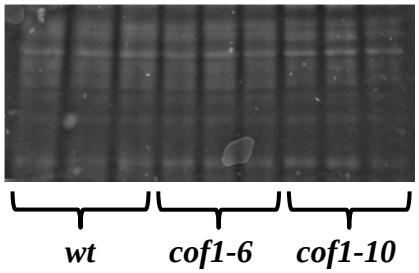
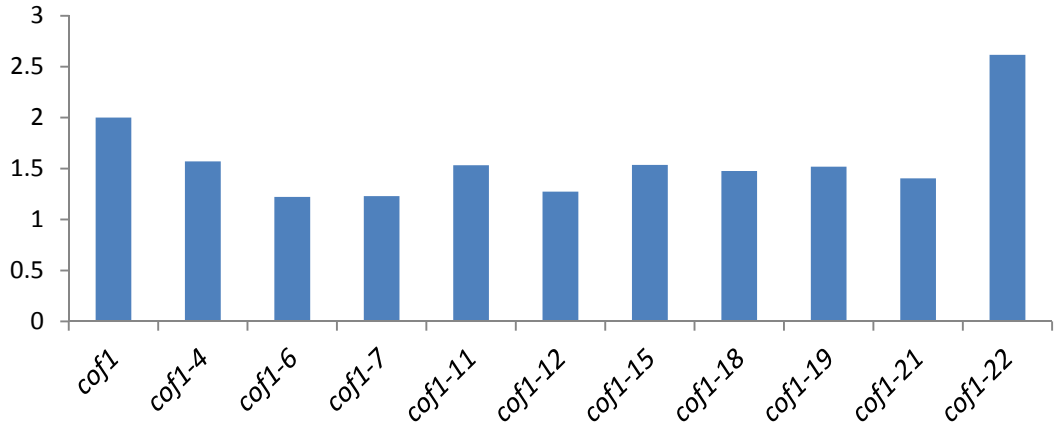
Strain	Routine	LEAK	ETS
Wild type	14.6 ±2.4	7.2±1.6	22.6±2.9
cof1-4	24.6±5.5	15.7±4.6	33.9±6.1
cof1-6	25.7±2.9	21±3.1	42.2±2.9
cof1-7	25.0±3.9	20.35±3.0	44.75±4.3
cof1-11	22.1±4.0	14.4±2.9	38.3±4.2
cof1-12	22.4±3.5	17.6±3.7	38.8±5.5
cof1-15	16.6±0.9	10.8±0.4	27.9±1.4
cof1-18	19.9±2.6	13.5±1.6	33.3±3.3
cof1-19	20.7±3.9	13.6±1.6	31.6±4.8
cof1-21	17.1±8.1	12.2±8.1	26.7±15.0
cof1-22	16.7±7.6	6.4±3.1	18.0±7.1

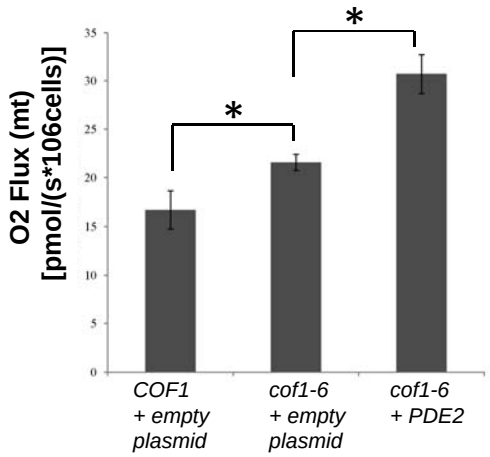
S1. High resolution respirometry was conducted on wild type and cofilin mutant strains grown for 24h to the diauxic shift phase of growth. Routine, LEAK (by addition of TET, 150 µM) and ETS (by addition of FCCP, 12 µM) respiration were measured in triplicate. Average values and their standard deviations are presented.



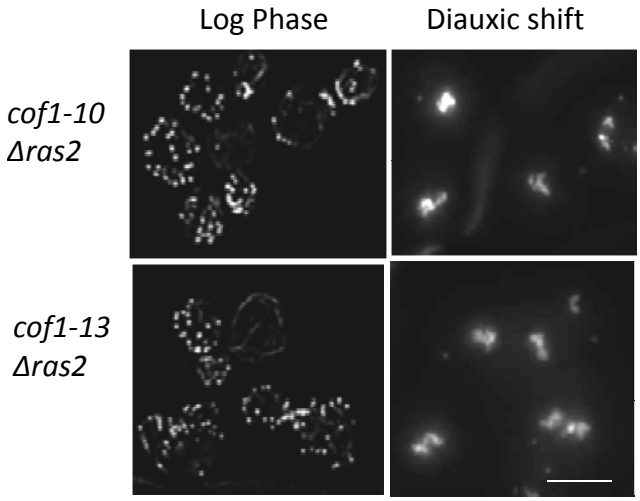
S2. Inverted image of Coomsaie stained gel utilised for western blot of Cox2p shown in Figure 3D following transfer to PVDF membrane. Proteins were prepared from precise and equivalent cell numbers according to the quantitative extraction method previously described (von der Haar T. PLoS One. 2007).



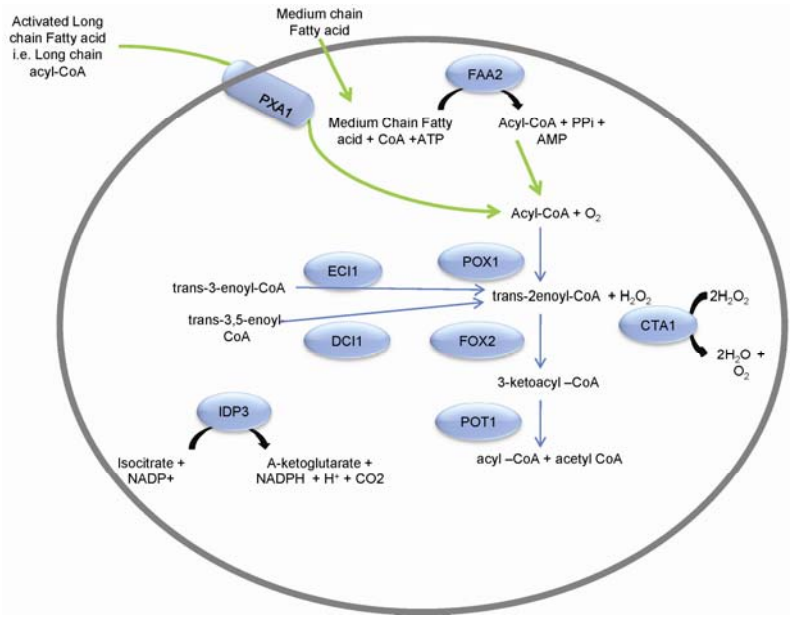
S3. A ratio of routine/LEAK respiration was generated for the averaged triplicate values of wild type and cofilin mutants grown for 24h to diauxic shift in YPD.



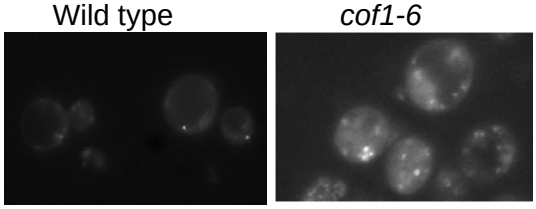
S4. *PDE2* was overexpressed in *cof1-6* expressing cells and cells grown for 24h to diauxic shift in YPD were examined for routine levels of respiration. Experiments were carried out in biological triplicate. Statistical significance was verified by a student TTEST ($P < 0.05$ *)



S5. F-actin was stained using rhodamine phalloidin in fixed wild type, *cof1-10Δras2* and *cof1-13Δras2* cell grown to either log or diauxic shift in YPD (E). Size bar = 10µm

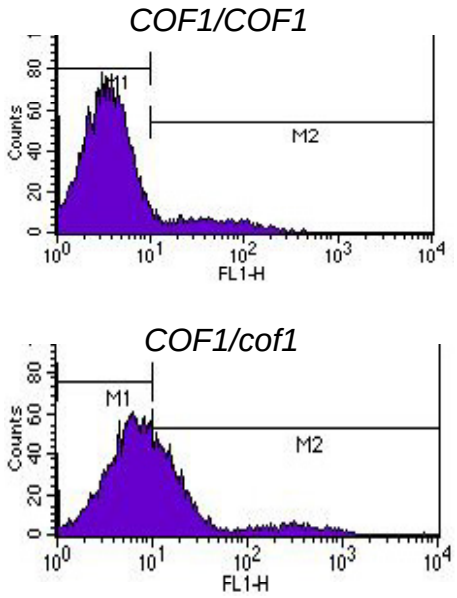


S6. Microarray analysis showed that the all of genes that required for the process of peroxisomal fatty acid β-oxidation were upregulated in *cof1-6* when compared to wild type cells, the up-regulated genes and the metabolic pathway are presented .

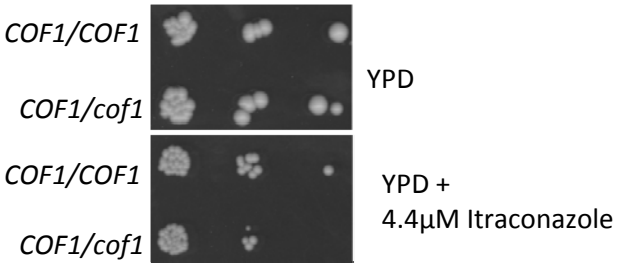


GFP-PTS1

S7. To examine the regulation of *CTA1* transcription in wild type and *cof1-6* cells we employed a construct containing a GFP-PTS1 reporter upstream of the *CTA1* promoter and examined the resultant fluorescence levels by microscopy (G).



S8. Cells were for 24 hours to diauxic shift in YPD. Following ROS accumulation was assessed in wild type (*COF1/COF1*) and heterozygous (*COF1/cof1*) strains using the indicator dye H_2DCF-DA by flow cytometry. Cells that exhibit fluorescence and so are judged to be under increased oxidative stress are detected in the M2 region on the representative histograms above.



S9. The effect of cofilin gene dosage on itraconazole sensitivity was tested by comparing growth of a wild type (*COF1/COF1*) and heterozygous (*COF1/cof1*) strain on YPD or YPD + 4.4µM Itraconazole containing agar plates.

name	Genotype	Cofilin Mutation	Source
CGY384	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 COF1::LEU2*	Wild type	Lappalainen *et al* , 1997
CGY385	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-4::LEU2*	S4A	Lappalainen *et al* , 1997
CGY386	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-5::LEU2*	D10A, E11A	Lappalainen *et al* , 1997
CGY387	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-6::LEU2*	D18A. K20A	Lappalainen *et al* , 1997
CGY388	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-7::LEU2*	C62A	Lappalainen *et al* , 1997
CGY389	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof-10::LEU2*	K42A, E43A	Lappalainen *et al* , 1997
CGY391	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-11::LEU2*	D47A, E51A	Lappalainen *et al* , 1997
CGY392	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-12::LEU2*	E55A, G56A	Lappalainen *et al* , 1997
CGY393	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-13::LEU2*	E59A, D61A	Lappalainen *et al* , 1997
CGY394	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-15::LEU2*	E77A, K79A	Lappalainen *et al* , 1997
CGY395	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-18::LEU2*	K105A, D106A	Lappalainen *et al* , 1997
CGY396	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-19::LEU2*	R109A, R110A	Lappalainen *et al* , 1997
CGY397	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-21::LEU2*	D130A	Lappalainen *et al* , 1997
CGY398	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-22::LEU2*	E134, R135A, R138A	Lappalainen *et al* , 1997
CGY904	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-10::LEU2 RAS2::HIS3*	K42A, E43A	This study
CGY905	*Matα ura3-52 his3Δ200 leu2-3,112 lys2-801 ade2-101 cof1-13::LEU2 RAS2::HIS3*	E59A, D61A	This study
CGY906	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0	Wild type	Open biosystems
CGY907	MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0 *COF1/cof1::KanMx*	*COF1/cof1*	Open biosystems

Table S1 – Strains used in this study

Table 2 – Supplemental Data

Go Term component	Genes
Cytoplasm	CYS3, GAL7, HBN1, YDL085C-A, ADH4, RSM27, LYS1, GRX8, YNR014W, ZPS1
Membrane	DAL3
Nucleus	HBN1, YDL085C-A, SNU23, MIF2
plasma membrane	ZRT1, DUR3, MEP2
mitochondrion	ADH4, RSM27
cell wall	ZPS1
Cellular component Unknown	YDR034C-A, YNL018C, YNL034W, YOR378W
Other	GAL10

Table S2 – Genes statistically significantly downregulated in *cof1-6* cells when compared to wild type by affymatrix microarray analysis. Genes were grouped by the gene ontology (GO) component analysis by the GO Slim mapper tool within the Saccharomyces Genome Database (SGD)(http://www.yeastgenome.org/cgi-bin/GO/goSlimMapper.pl) .

Table 3 supplemental data

Go Term	Gene
cytoplasm	FUS3,CHS3,ADY2,SRD1,SNQ2,CTA1,ATO3,FAA2,POX1,GND2,YKE4,VHR1,RGI 2,POT1,IMA3,INO1,FAR1,REE1,TPO1,ECI1,RPS22B,YML131W,ALD2,ADH2, DIA1,ERR3,IDP3,GRE2,RSB1,PDR5,DCI1,FDH1,PMA2,RPS9A,PXA1,CIT3
membrane	CHS3,ADY2,SNQ2,ATO3,PDR15,RTA1,YOR1,YHR140W,DAL4,FAR1,TPO1, FRE7,RSB1,PDR5,MCH5,PDR1PMA2,PXA1,AQY1
plasma membrane	ADY2,SNQ2,ATO3,YOR1,TPO1,FRE7,RSB1,PDR5,MCH5,PDR1PMA2,AQY1
mitochondrion	FUS3,ADY2,SNQ2,CTA1,ATO3,FAA2,IDP3,PDR5,PMA2,CIT3
nucleus	FUS3,TEC1,SRD1,YHL009W-A,YHL009W-B,VHR1,FAR1,NDJ1,GRE2
peroxisome	CTA1,FAA2,POX1, FOX2, POT1,ECI1,IDP3,DCI1,PXA1
membrane fraction	ADY2,HBT1,SNQ2,PAU5,GND2,YOR1,PMA2
Cell Wall	SPS100,SIM1,PLB2,FIT2
Site of polarised growth	FUS3,CHS3,FAR1
riboosome	RPS22B, RPS9A
Endoplasmic reticulum	YKE4, RSB1
Extracellular region	PLB2
Chromosome	NDJ1
Cellular component unknown	YBR013C,YCL021W-A,DDI2,YGR035C,IMA5,ICT1,YOR032W-A,PAU21,YPL088W,YPL113C
Other	BAG7

Table S3 – Genes statistically significantly upregulated in *cof1-6* cells when compared to wild type by affymatrix microarray analysis. Genes were grouped by the gene ontology (GO) component analysis by the GO Slim mapper tool within the Saccharomyces Genome Database (SGD) (http://www.yeastgenome.org/cgi-bin/GO/goSlimMapper.pl) .

Table 4 supplemental data

Primer	Nucelotide Sequence 5'-3'
RAS2 deletion Forward	ATGCCTTTGAACAAGTCGAACATAAGAGA GTACAAGCTAGTCGTCGTTGGCGGATCC CCGGGTTAATTAA
RAS2 deletion reverse	CCACCCGATCCGCTCTTGGAGGCTTCAC TGGTGTTACCGCCGGGTGCAGCGAATTC GAGCTCGTTTAAAC
PDR1 deletion forward	ATGCGAGGCTTGACACCTAAGAACGGTG TACATATTGAGACGGGCAGTTGAAGCTTC GTACGC
PDR1 delete reverse	TTAACTATCTGGATAAACGTCGCTCCACA GGATACTGTAGAGGTCGCATAGGCCACTA GTGGATCTG
PDR3 delete forward	ATGAAAGTGAAGAAATCAACTAGATCAAA AGTTTCGCACGCATGCAGCTGAAGCTTC GTACGC
PDR3 delete reverse	CATAAGAAGGGATATGAAGTATTGTCATTC CACAGAGTATGATAGCATAGGCCACTAGT GGATCTG

Table S4 - Primers used for gene deletion in this study