Supporting information for

Proteome-wide tagging with an H_2O_2 biosensor reveals highly localized and dynamic redox microenvironments

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[5]Equal contribution

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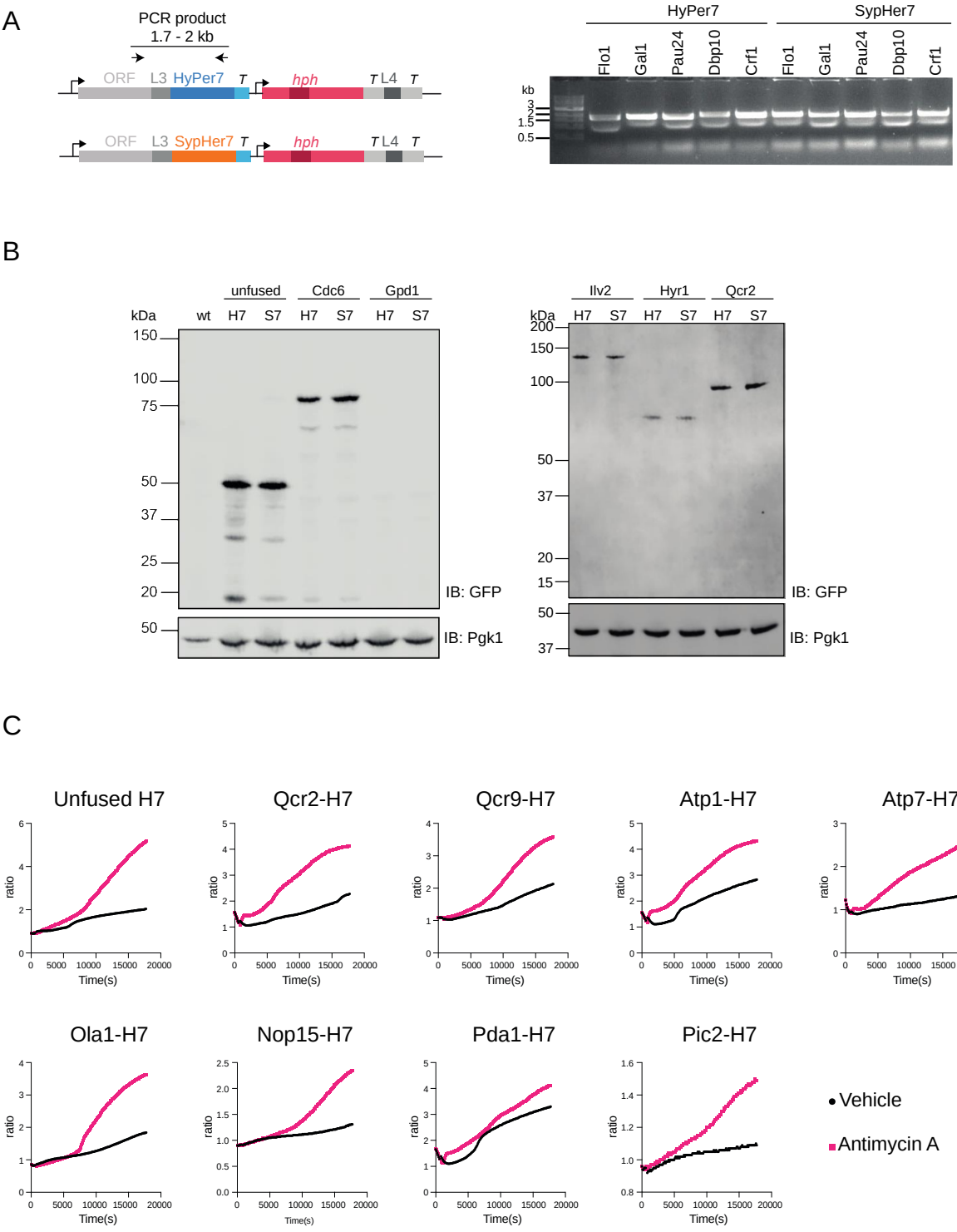
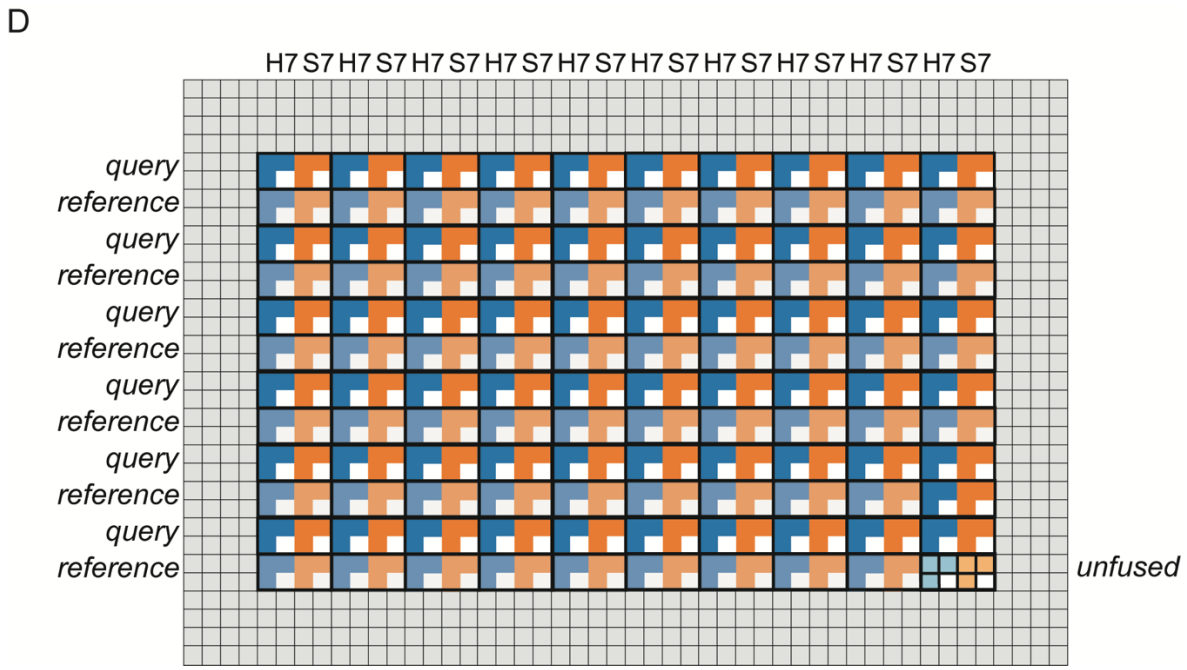
This PDF files includes Supplementary Figures S1-S7.

Other supporting materials for this manuscript include the following: Datasets S1-S3


A
PCR product
1.7 - 2 kb
ORF L3 HyPer7 T hph T L4 T
ORF L3 SypHer7 T hph T L4 T
HyPer7
SypHer7
Flo1
Gal1
Pau24
Dbp10
Crf1
kb
3
2
1.5
0.5
B
unfused
Cdc6
Gpd1
kDa
wt
H7
S7
150
100
75
50
37
25
20
IB: GFP
IB: Pgk1
Ilv2
Hyr1
Qcr2
200
15
C
Unfused H7
Qcr2-H7
Qcr9-H7
Atp1-H7
Atp7-H7
Ola1-H7
Nop15-H7
Pda1-H7
Pic2-H7
ratio
Time(s)
Vehicle
Antimycin A



Supplementary Figure S1

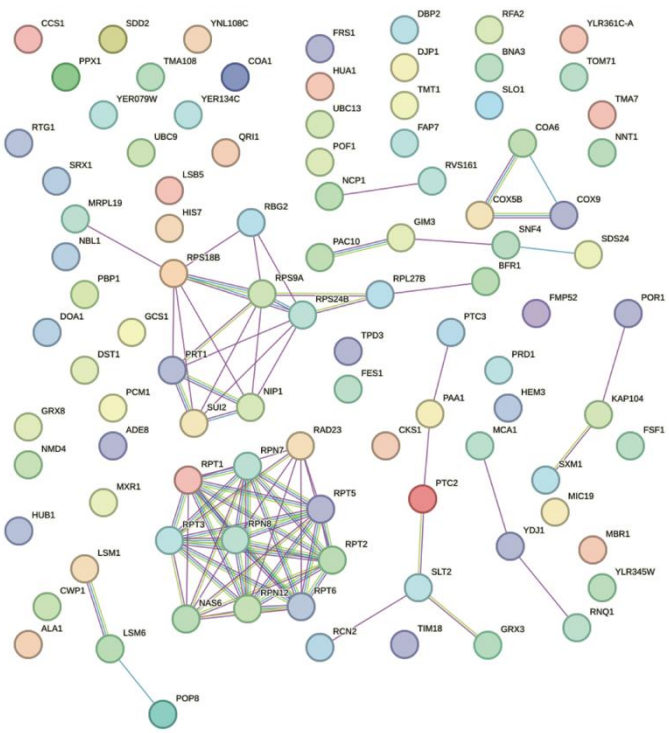
(A) Validation of genomic tagging. Left panel: PCR amplification strategy. Right panel: Results for five randomly selected strains.

(B) Validation of fusion protein expression using anti-GFP immunoblotting. Paired strains (HyPer7/SypHer7) covering a range of expression levels are shown.

(C) Response of selected HyPer7 fusion proteins to antimycin A. Upper panels: ETC fusion proteins (Atp1, Atp7: ATP synthase subunits; Qcr2, Qcr9: complex III subunits). Lower left panels: cytosolic and nuclear fusion proteins (unfused HyPer7; Ola1: P-loop ATPase; Nop15: 66S pre-ribosomal particle nucleolar protein). Lower right panels: non-ETC mitochondrial fusion proteins (Pda1: pyruvate dehydrogenase subunit E1; Pic2: mitochondrial phosphatase and copper carrier).

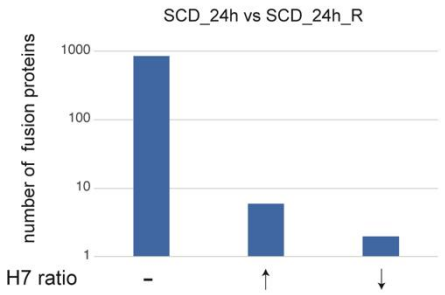
(D) Library plate layout. Each 2x4 unit (black outline) contains three HyPer7 (H7; blue) and three SypHer7 colonies (S7; orange), together representing one fusion protein. One non-fluorescent strain (white) is included for local background correction. Each plate contains 60 different HyPer7/SypHer7 strains (fusion proteins). Each plate also contains 59 paired (HyPer7/SypHer7) reference strains (light blue and light orange, respectively) and triplicates of strains expressing unfused HyPer7/SypHer7 (light blue and orange, respectively, with additional black outline, lower right corner).

A



Biological Process (Gene Ontology)				
GO-term	*description*	*count in network*	*strength*	*false discovery rate*
GO:0070682	Proteasome regulatory particle assembly	6 of 11	1.57	0.00023
GO:0045899	Positive regulation of RNA polymerase II tr...	5 of 11	1.49	0.0016
GO:0043161	Proteasome-mediated ubiquitin-dependen...	14 of 160	0.77	0.00027
GO:0051247	Positive regulation of protein metabolic pr...	11 of 145	0.71	0.0035
GO:0051603	Proteolysis involved in protein catabolic pr...	15 of 262	0.59	0.0033
GO:0043632	Modification-dependent macromolecule c...	15 of 261	0.59	0.0033
GO:0051246	Regulation of protein metabolic process	21 of 405	0.55	0.00050
GO:0044265	Cellular macromolecule catabolic process	18 of 381	0.51	0.0035
GO:0006508	Proteolysis	17 of 360	0.51	0.0047
GO:0009057	Macromolecule catabolic process	19 of 446	0.46	0.0055
GO:1901565	Organonitrogen compound catabolic proc...	17 of 405	0.46	0.0159
GO:0019538	Protein metabolic process	43 of 1303	0.35	0.00023
GO:1901564	Organonitrogen compound metabolic proc...	47 of 1817	0.25	0.0034
GO:0043170	Macromolecule metabolic process	53 of 2379	0.18	0.0251
GO:0009987	Cellular process	91 of 4883	0.1	0.00050

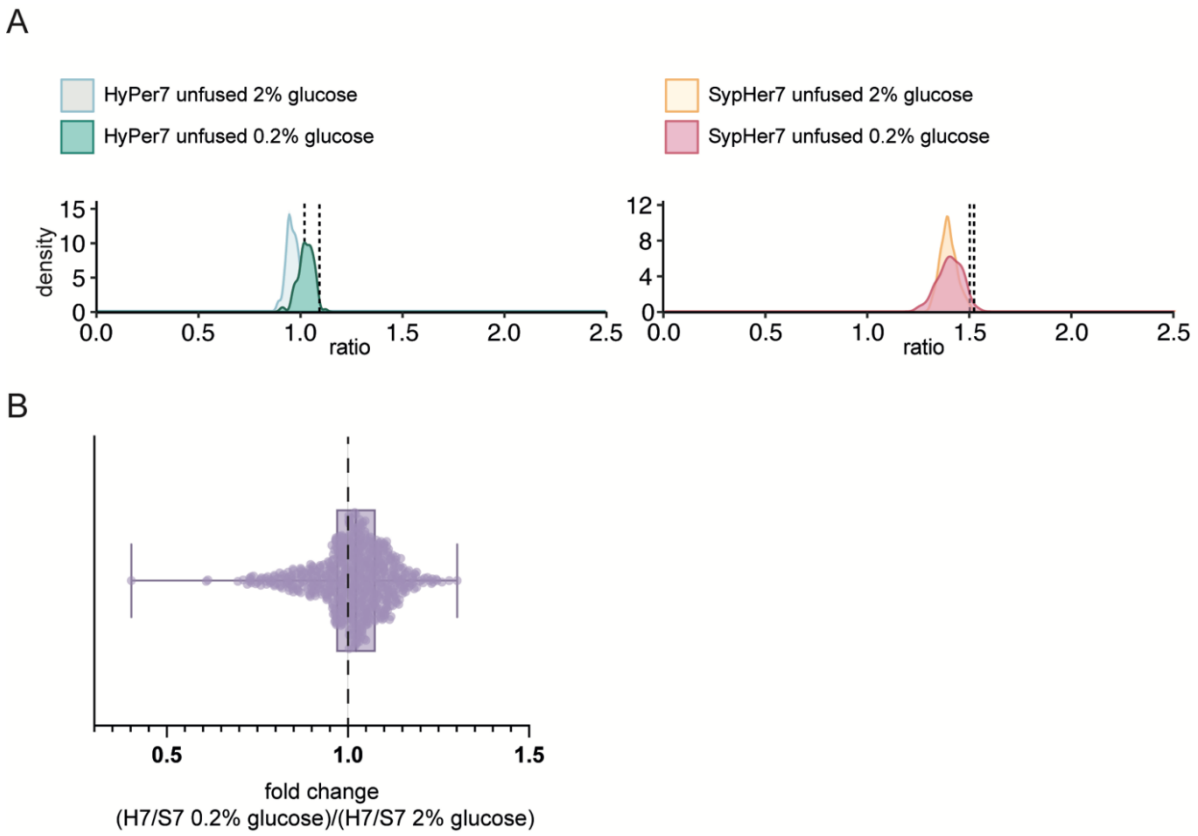
B



Supplementary Figure S2

(A) Proteins exhibiting increased ratios in both HyPer7 and SypHer7 fusion libraries for SCD_24h. Upper Panel: STRING interaction network. Lower panel: GO term enrichment. The list of fusion proteins with elevated HyPer7 and SypHer7 ratios in all conditions can be found in Dataset S1.

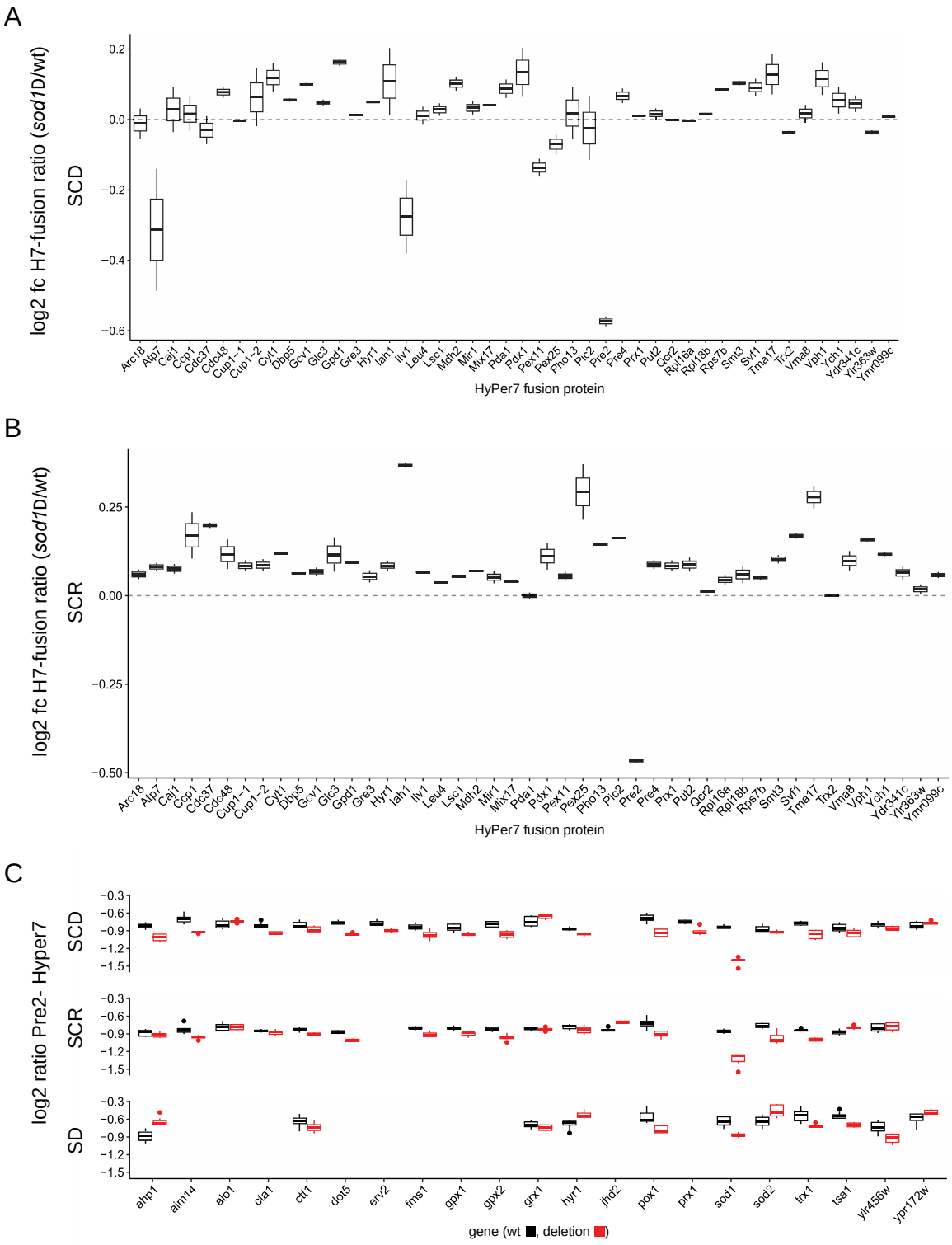
(B) Comparison between SCD_24h and SCD_24h_R. Number of fusion proteins with (↑,↓) or without (-) a significant ($p<0.05$) difference in the HyPer7 (H7) ratio. Proteins exhibiting an altered SypHer7 ratio were excluded from the analysis.

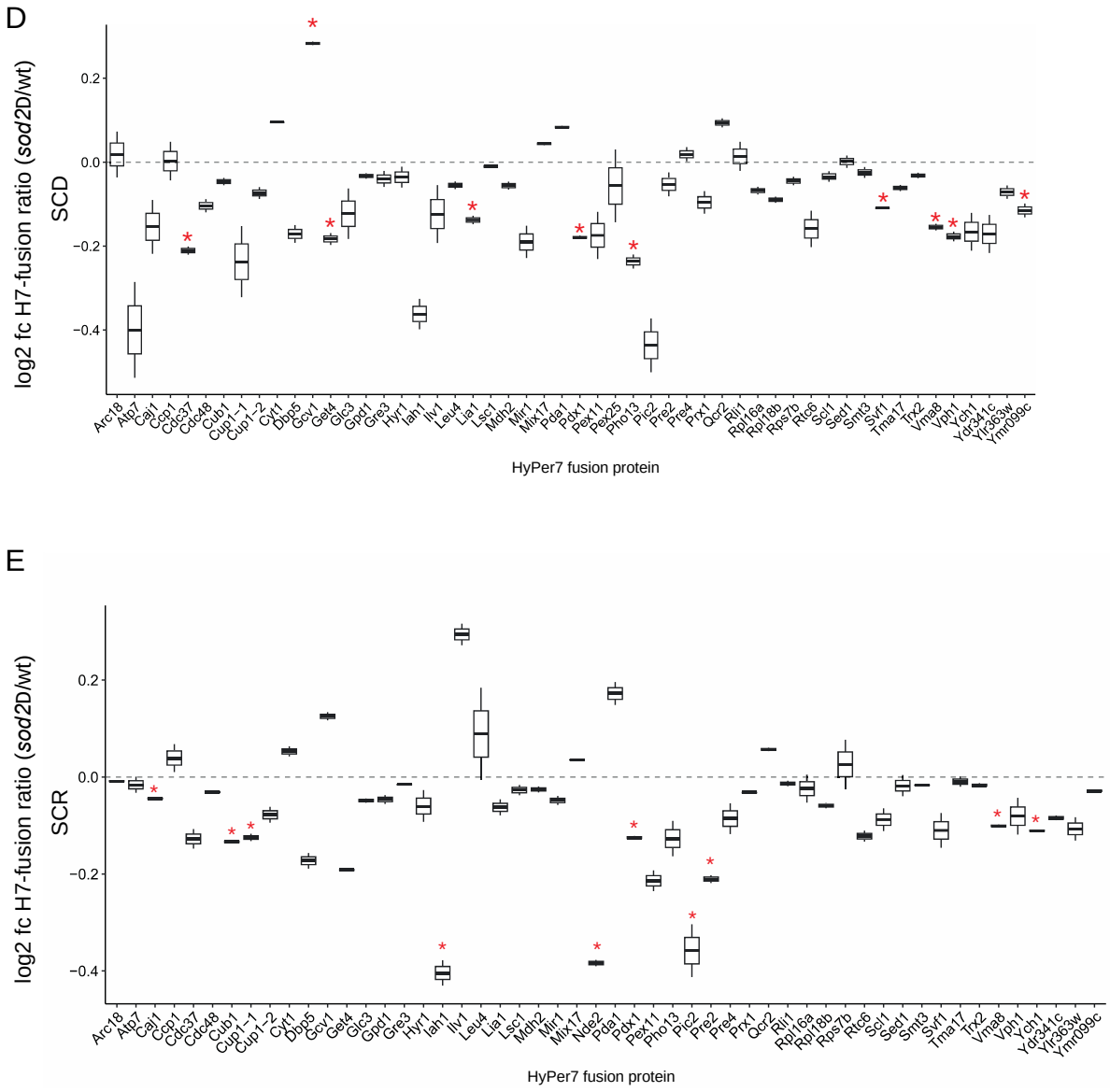


Supplementary Figure S3

(A) Left panel: Ratio distributions for free (unfused) cytosolic HyPer7 as measured on 2% (light blue) or 0.2% (teal) glucose SCD plates. Right panel: Ratio distributions for free (unfused) cytosolic SypHer7 as measured on 2% (light orange) or 0.2% (pink) glucose SCD plates.

(B) Plot of fold change (HyPer7/SypHer7 ratio in 0.2% glucose)/(HyPer7/Sypher ratio in 2% glucose) for the 674 fusion proteins with reliable signal in both 2% and 0.2% glucose. The majority of fusion proteins (417) is more oxidized (fc>1) under low glucose conditions.


A
log2 fc H7-fusion ratio (sod1D/wt)
SCD
0.2
0.0
−0.2
−0.4
−0.6
Arc18 Atp7 Caj1 Ccp1 Cdc37 Cdc48 Cup1−1 Cup1−2 Cyt1 Dbp5 Gcv1 Glc3 Gpd1 Gre3 Hyr1 Iah1 Ilv1 Leu4 Lsc1 Mdh2 Mir1 Mlx17 Pda1 Pdx1 Pex11 Pex25 Pho13 Pic2 Pre2 Pre4 Prx1 Put2 Qcr2 Rpl16a Rpl18b Rps7b Smt3 Svf1 Tma17 Trx2 Vma8 Vph1 Ych1 Ydr341c Ylr363w Ymr099c
HyPer7 fusion protein
B
log2 fc H7-fusion ratio (sod1D/wt)
SCR
0.25
0.00
−0.25
−0.50
Arc18 Atp7 Caj1 Ccp1 Cdc37 Cdc48 Cup1−1 Cup1−2 Cyt1 Dbp5 Gcv1 Glc3 Gpd1 Gre3 Hyr1 Iah1 Ilv1 Leu4 Lsc1 Mdh2 Mir1 Mlx17 Pda1 Pdx1 Pex11 Pex25 Pho13 Pic2 Pre2 Pre4 Prx1 Put2 Qcr2 Rpl16a Rpl18b Rps7b Smt3 Svf1 Tma17 Trx2 Vma8 Vph1 Ych1 Ydr341c Ylr363w Ymr099c
HyPer7 fusion protein
C
log2 ratio Pre2- Hyper7
SCD
SCR
SD
−0.3
−0.6
−0.9
−1.2
−1.5
ahp1 aim14 alo1 cta1 ctt1 dot5 erv2 fms1 gpx1 gpx2 grx1 hyr1 jhd2 pox1 prx1 sod1 sod2 trx1 tsa1 yir456w ypr172w
gene (wt ■, deletion ■)



Supplementary Figure S4

(A) Influence of Sod1 deficiency on the HyPer7 redox state in various fusion proteins on SCD (left panel). Significant changes ($p<0.05$) are marked in red.

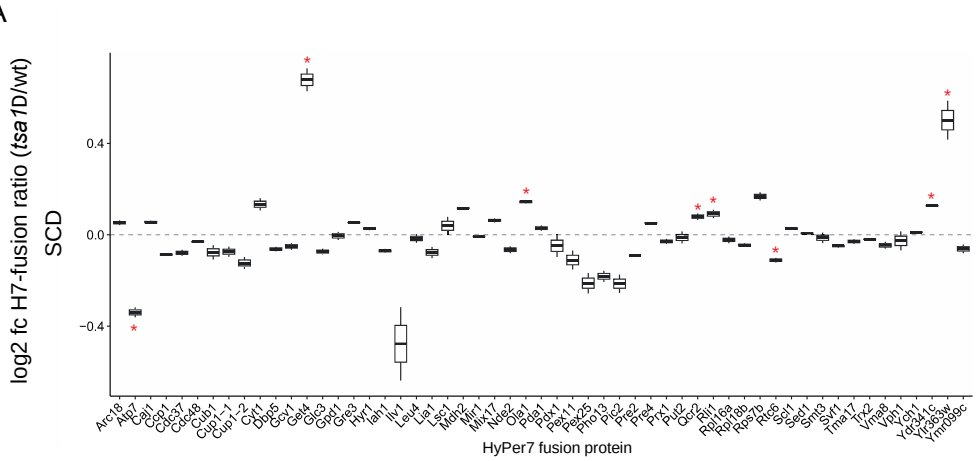
(B) Influence of Sod1 deficiency on the HyPer7 redox state in various fusion proteins on SCR (right panel). Significant changes ($p<0.05$) are marked in red.

(C) Response of the Pre2-HyPer7 redox state to various gene deletions under different growth conditions: SCD (top), SCR (middle) and SD (bottom). Wild type strains are shown in black. Deletion strains are shown in red.

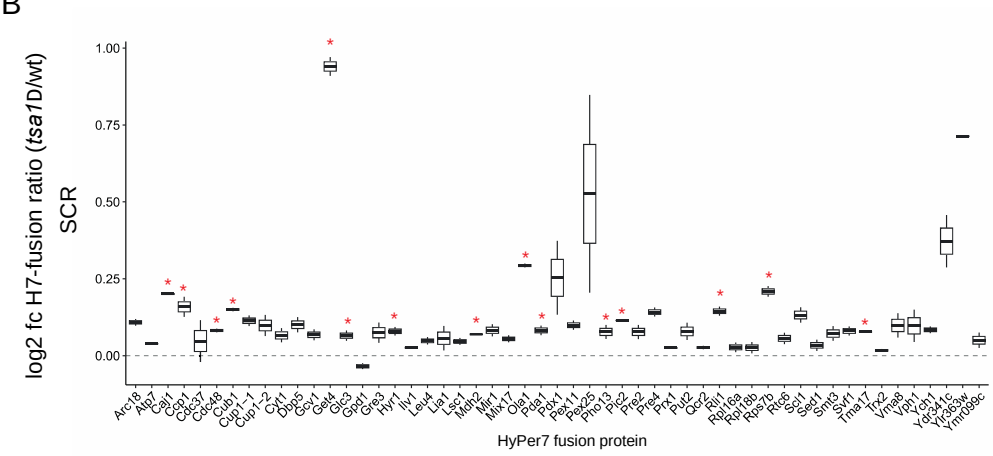
(D) Influence of Sod2 deficiency on the HyPer7 redox state in various fusion proteins on SCD (left panel) Significant changes ($p<0.05$) are marked in red.

(E) Influence of Sod2 deficiency on the HyPer7 redox state in various fusion proteins on SCR (right panel). Significant changes ($p<0.05$) are marked in red.

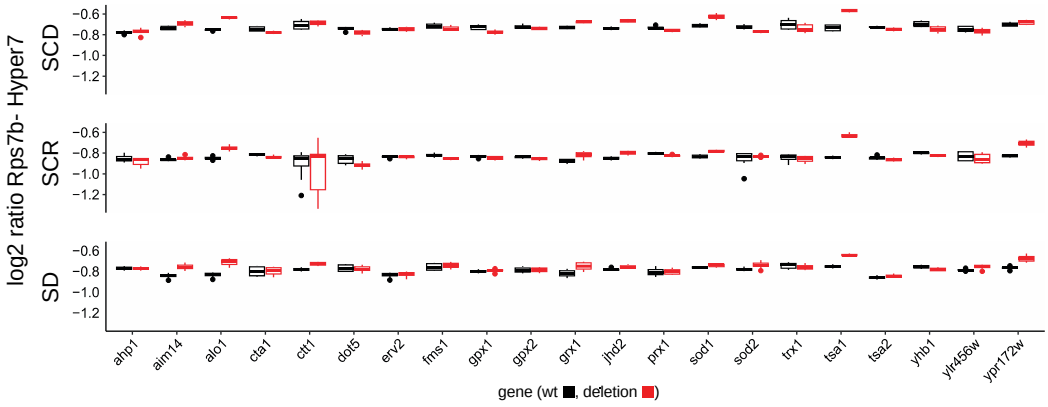
A



B



C

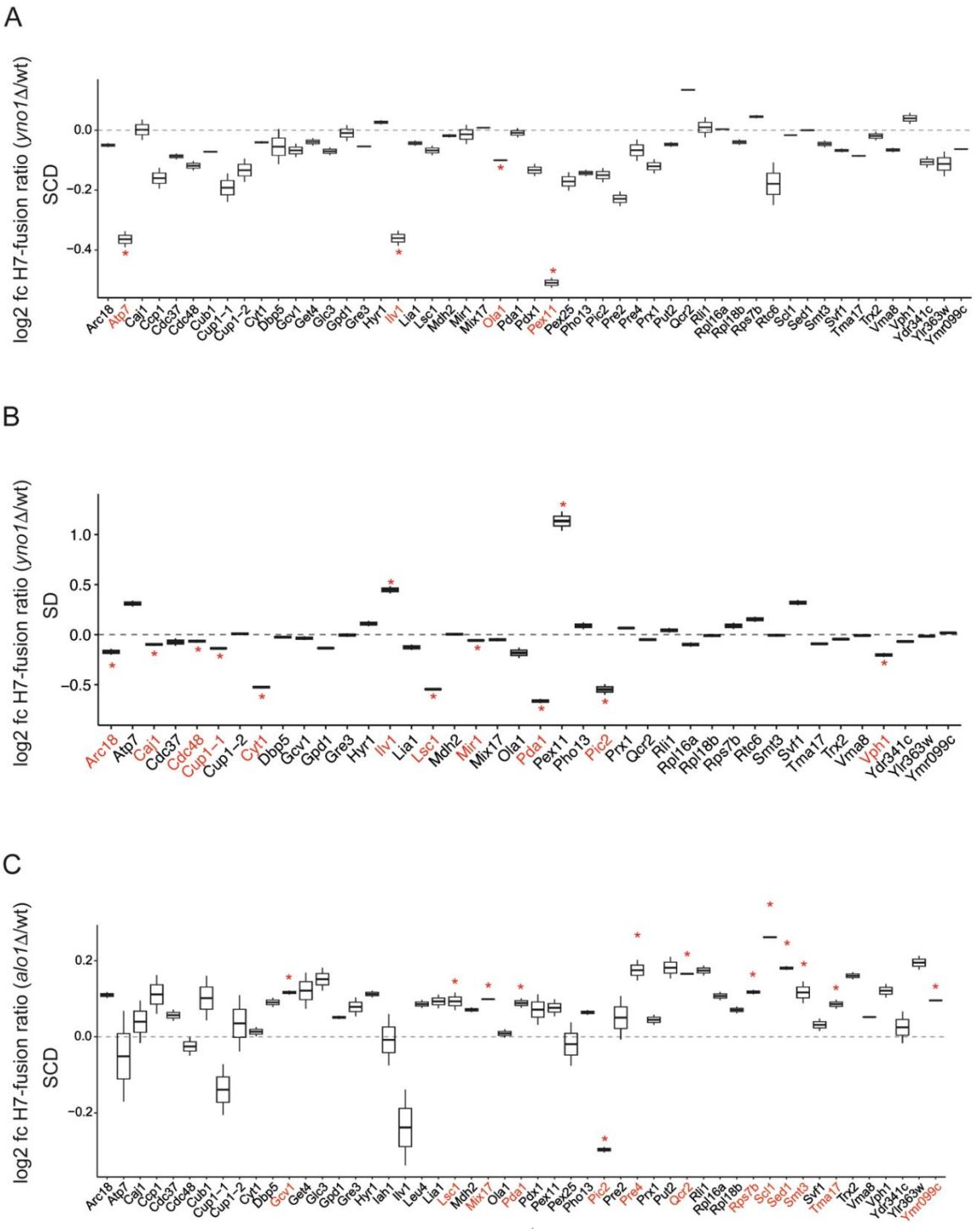


Supplementary Figure S5

(A) Influence of Tsa1 deficiency on the HyPer7 redox state in various fusion proteins on SCD (left panel). Significant changes ($p<0.05$) are marked in red.

(B) Influence of Tsa1 deficiency on the HyPer7 redox state in various fusion proteins on SCR (right panel). Significant changes ($p<0.05$) are marked in red.

(C) Response of the Rps7b-HyPer7 redox state to various gene deletions under different growth conditions: SCD (top), SCR (middle) and SD (bottom). Wild type strains are shown in black. Deletion strains are shown in red.

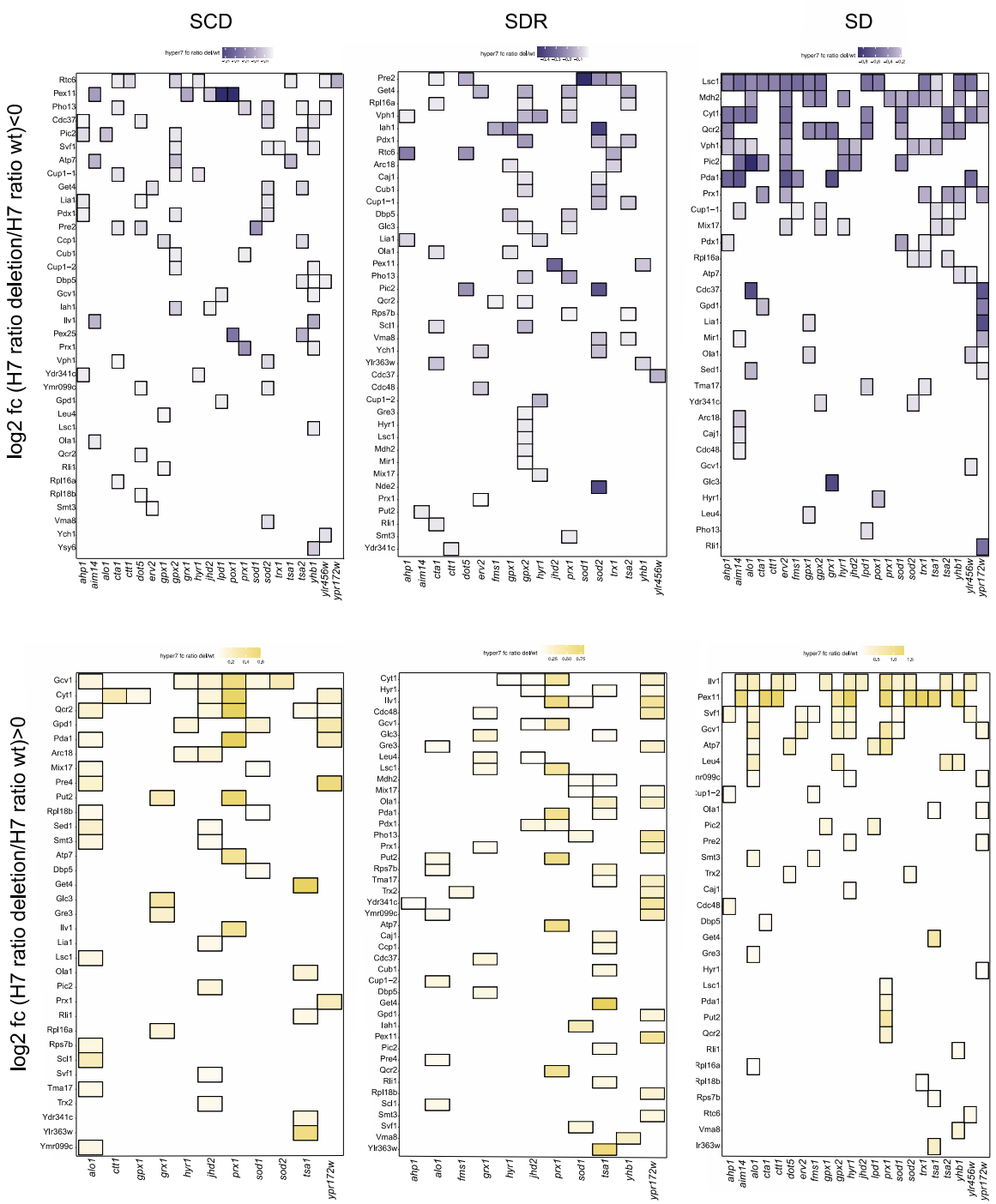


Supplementary Figure S6

(A) Influence of Yno1 deficiency on the HyPer7 redox state in various fusion proteins on SCD. Significant changes ($p<0.05$) are marked in red.

(B) Influence of Yno1 deficiency on the HyPer7 redox state in various fusion proteins on SD. Significant changes ($p<0.05$) are marked in red.

(C) Influence of Alo1 deficiency on the HyPer7 redox state in various fusion proteins on SCD. Significant changes ($p<0.05$) are marked in red.



Supplementary Figure S7

Heat maps summarizing all measured combinations of fusion proteins and deletions. Only significant changes ($p<0.05$) in the HyPer7 ratio are indicated. Upper panels: Deletions that lead to a decreased HyPer7 ratio (purple shades). Lower panels: Deletions that lead to an increased HyPer7 ratio (yellow shades). Strains were grown on SCD (left panels), SCR (middle panels), and SD (right panels).