end-to-end chromosome fusions and centromere deletions. The fusion of sixteen native linear chromosomes into a single chromosome results in marked changes to the global three-dimensional structure of the chromosome due to the loss of all centromere-associated inter-chromosomal interactions, most telomere-associated inter-chromosomal interactions and 67.4% of intra-chromosomal interactions. However, the single-chromosome and wild-type yeast cells have nearly identical transcriptome and similar phenome profiles. The giant single chromosome can support cell life, although this strain shows reduced growth across environments, competitiveness, gamete production and viability. This synthetic biology study demonstrates an approach to exploration of eukaryote evolution with respect to chromosome structure and function.

rme ia pil sula

1

m sapiens

2

untia us munt a

Sa har m es erevisiae

12

S hi sa har m es p m e

1

S. erevisiae

2 0 1 00

Rationale

S. erevisiae 2

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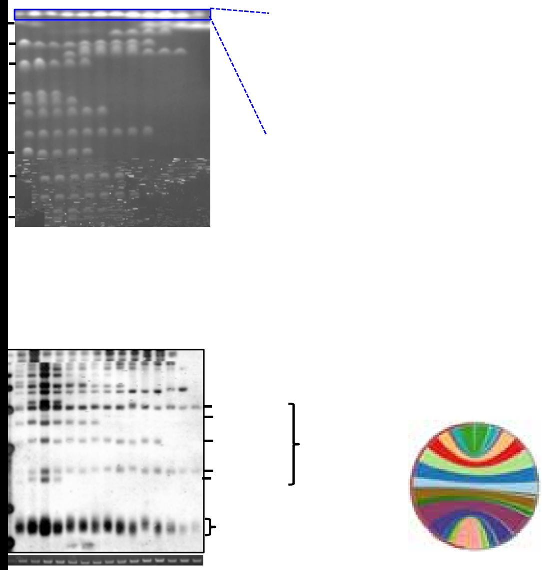
1 2

[redacted]



1

1 [11]

2

S 1

S 1

2 2

2 S 1

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omosomal 3D structures

S. erevisae [12 1]

2 S 1

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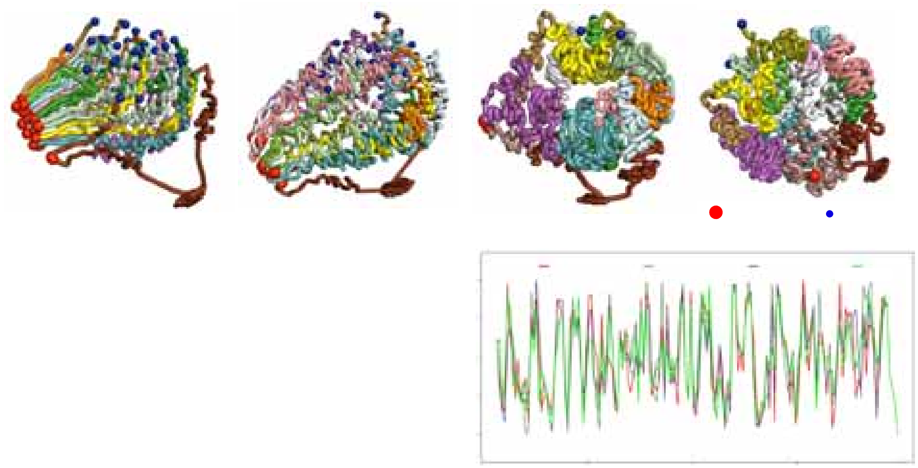
2 [1 1]

2 S S 1 S 1



2
0 01 0 01

S 1
12 S 1
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S 12

S 1 S 1 S 1 1

2
S S 1 S 1
2 S 0 0 2 2 10 [1]

S 1

2 S

Transcriptome and phenome analysis

2 S 1

2
2 S S 1 S 1 2
S 1 S 28 S 1 2
0 81
2 [1]

18 1
0 AL11 L1 3 L1 2 S 1
1
8 0 01 0 01 S 1 *2 S 32 2 L2*
2 *3* S 1
S 1 S 1

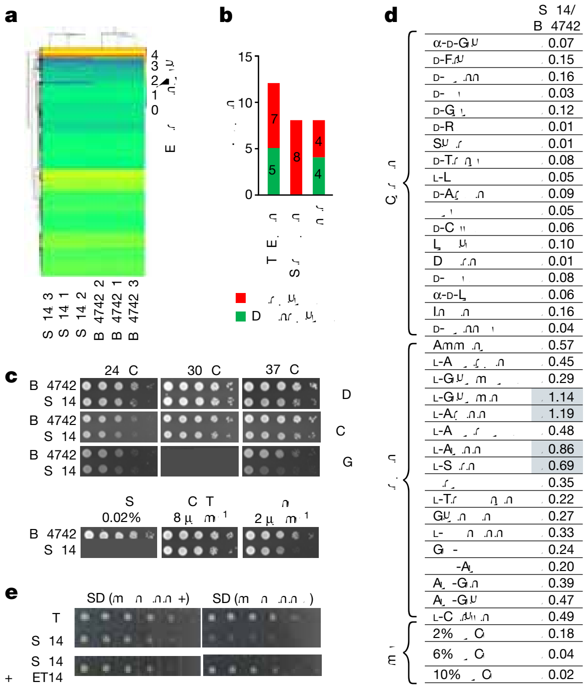

Fig. 4 Transcriptome and phenome analyses. a

2 S 1

n 0 8 0

b

^{2} 1 0 001

S 1 **c** S 1

d 2 S 1

S 1

2

2

0 0 1 2 2 8 12

2

S 1

S 1

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S

S 1

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S 1

1

S 1

^{2}

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1

2 S 1

S 1

2 S 1

S 1 2

S 1

Meiosis and spore viability

2 S 1

[20]

S 1 S 1

S 1 S 1

S 1 S 1

S 1 S 1

2 2

S 1 S 1 2 2

S 1 S 1

S 1 S 1 8

8 2 2

S S S 1 S 1

Discussion

S. erevisiae [1,21]

S. erevisiae

S 1

22 2

S 1

S 1 2

S. erevisiae

12 2

11 8

S. erevisiae[2] S 1 S 1

S. erevisiae

10 20

2

2

S 0 S 1

Online content

S 28 0 1 8 1

METHODS

Plasmid constructions.
28 *1* *L* *2*
1
S 2 2 *S3*
S. *erevisae* S288 2
S 2 1 S 2
SN *2* 20
S 20
1 2

S 2
S
2 S 2 1 S
80
20
1 2

S 2

CRISPR–Cas9 facilitated chromosome fusion. 1
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S. *erevisae* 2

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S

S 0
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S

Telomere Southern blot. S
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+

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81 1 1 8 S 2 1 2 0 8 1 2 2 11 1 2 2 10 0 12 0 0

1 8
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0 02 S 8 0 01
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0 S S 0 12 S
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S 8 0 01
100 12
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2 01

Cell growth, morphology and cell cycle analysis. S 2 S 1

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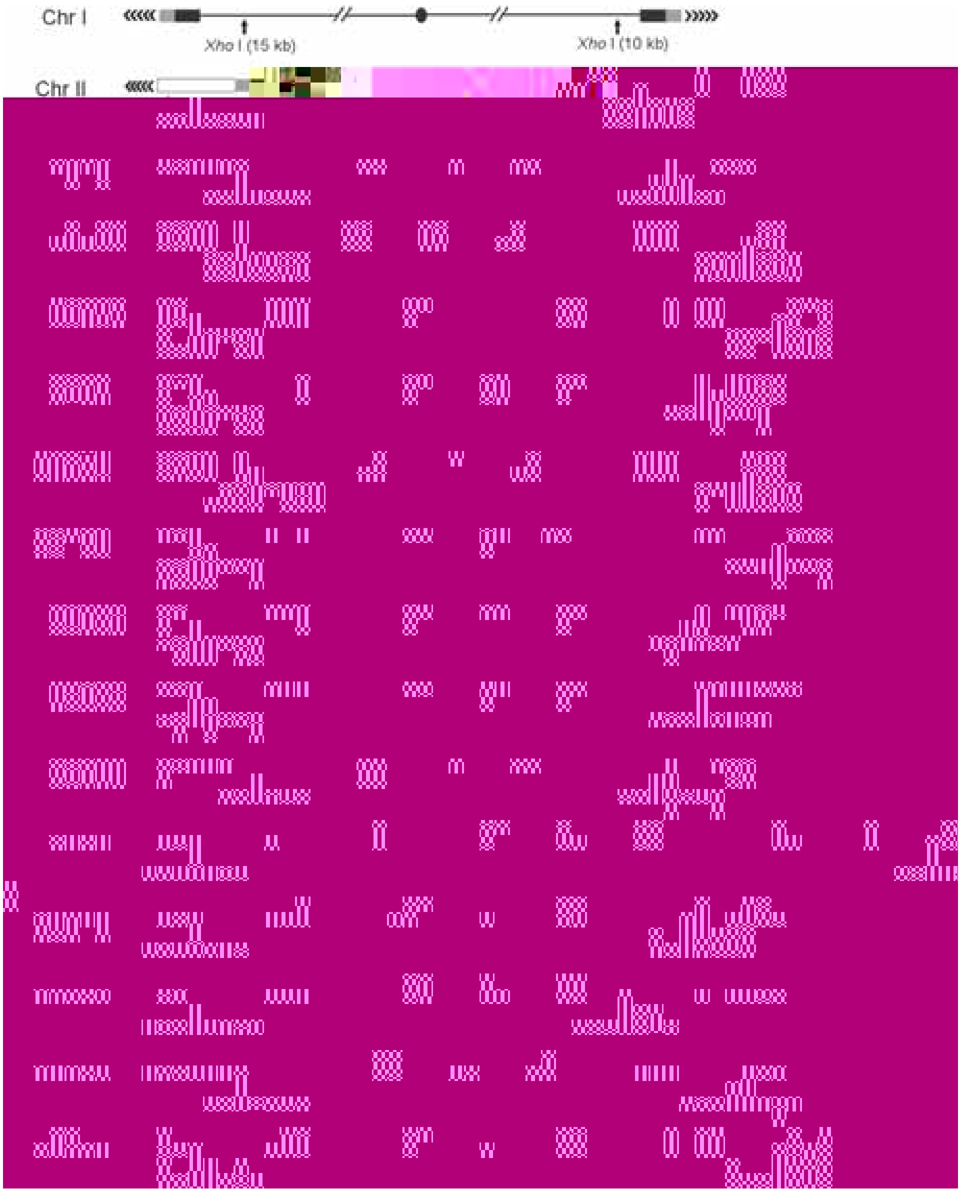
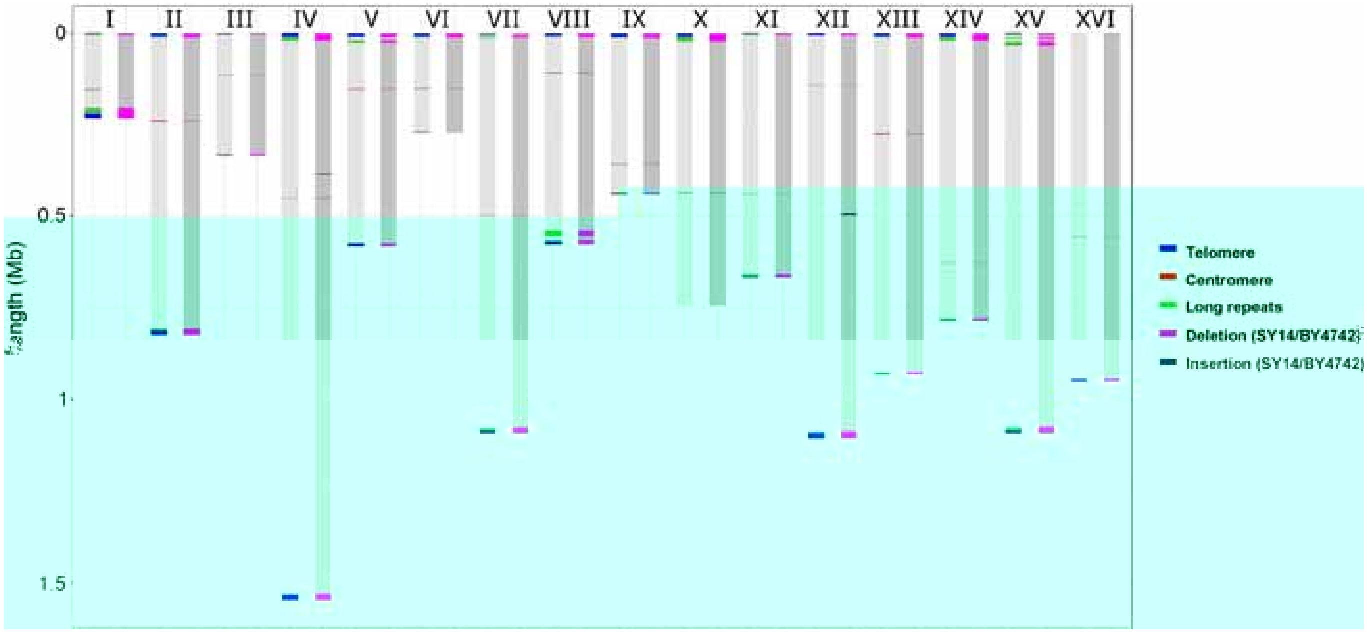
200 1

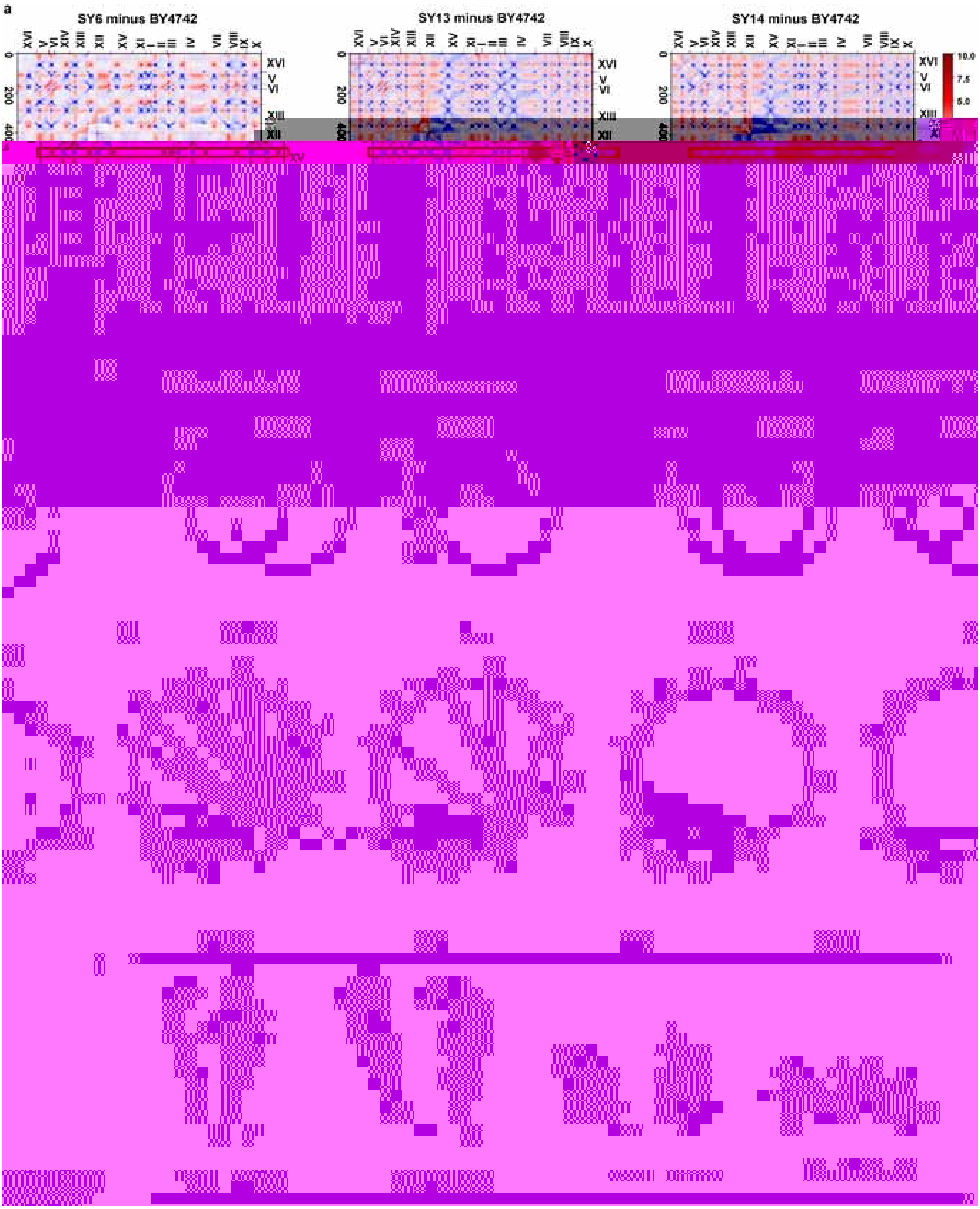


Extended Data Fig. 1 Theoretical XhoI digestion pattern of chromosome ends. S 1 S 1



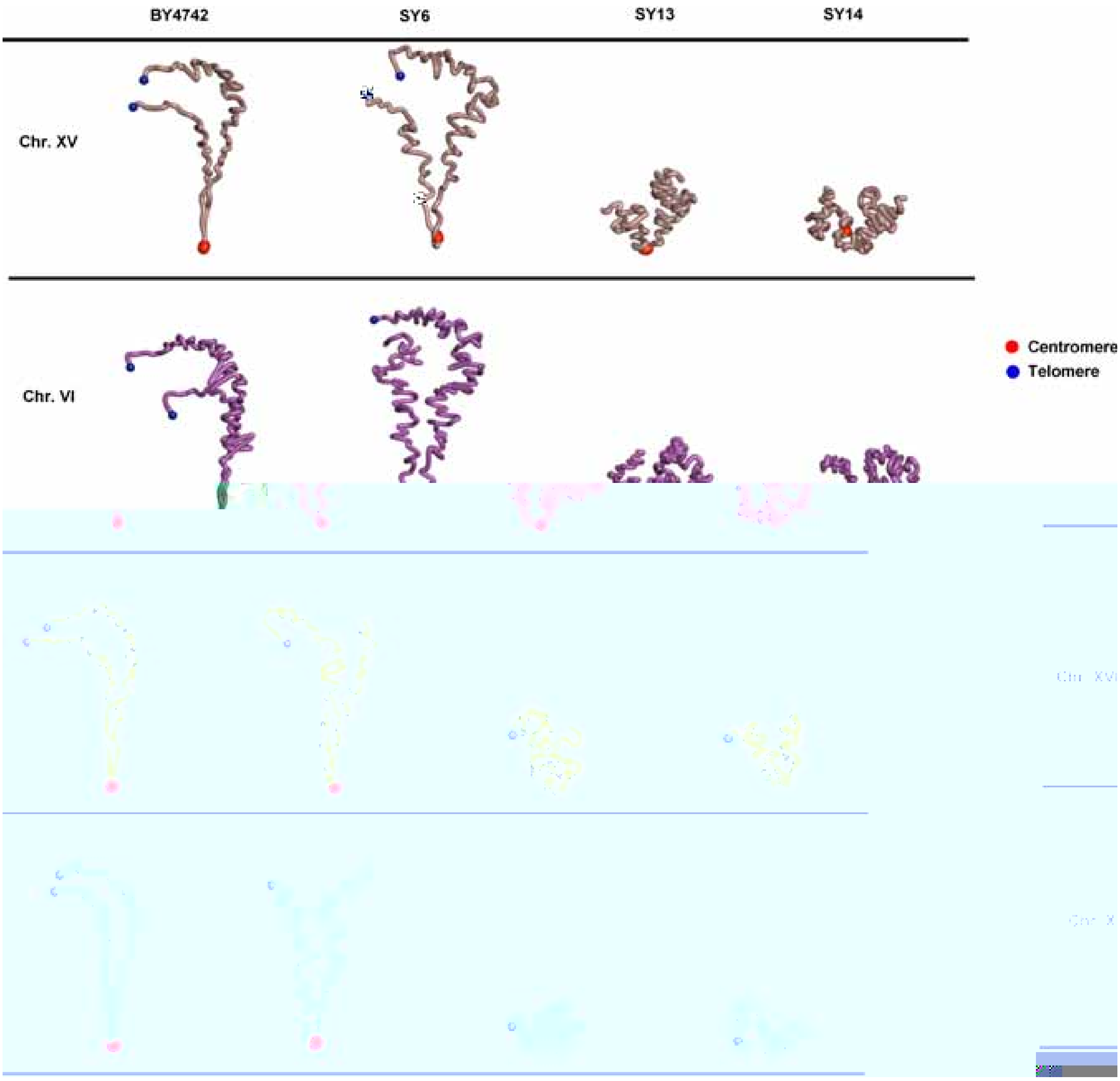
Extended Data Fig. 2 De novo sequence comparison of BY4742 (light grey) and SY14 (dark grey) genomes. 2 S

2 S 1
S 1

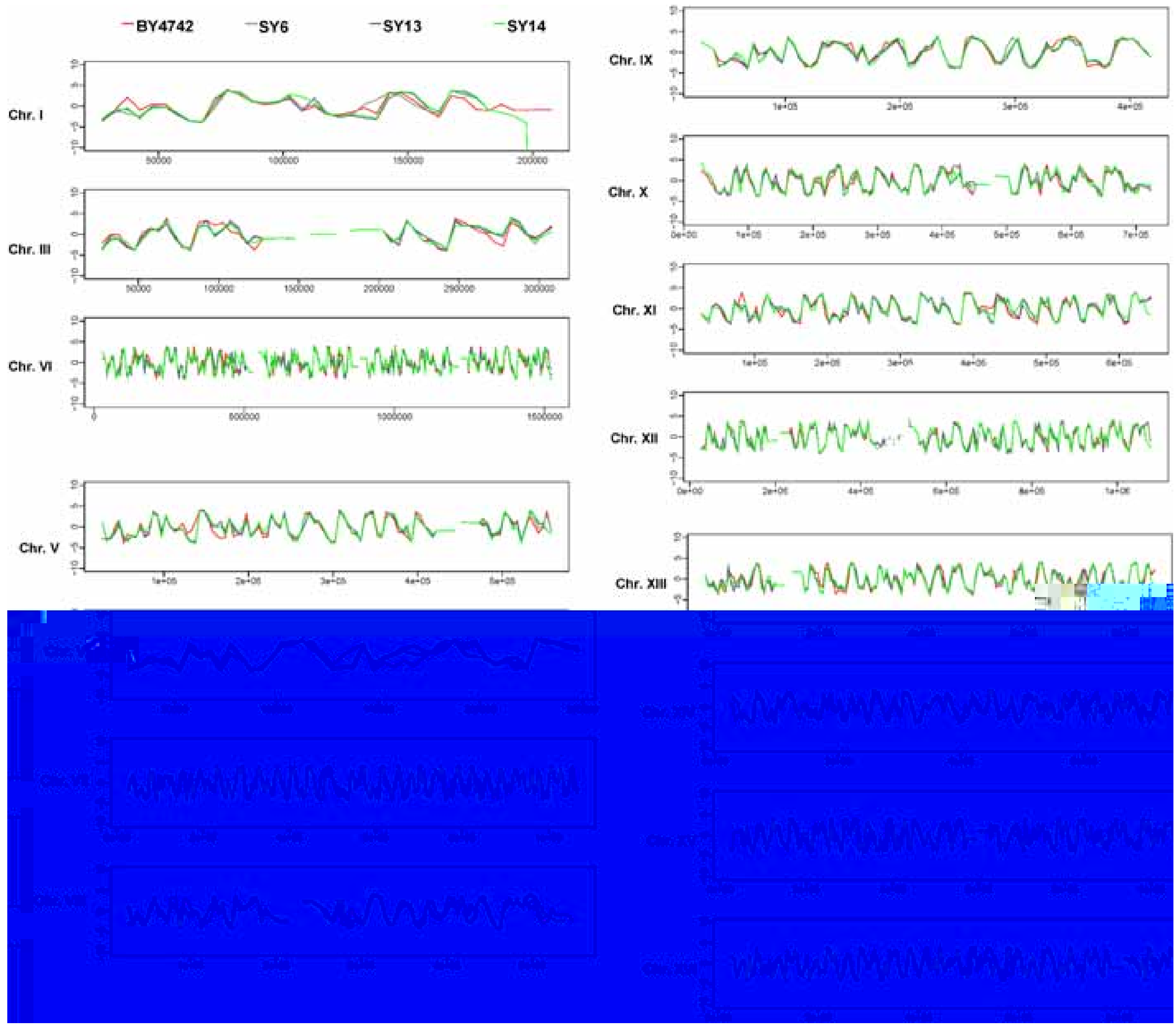


Extended Data Fig. 3 Comparison of the chromosomal interactions of SY6, SY13 and SY14 cells with those of BY4742 cells. a 0 10 S S 1 S 1

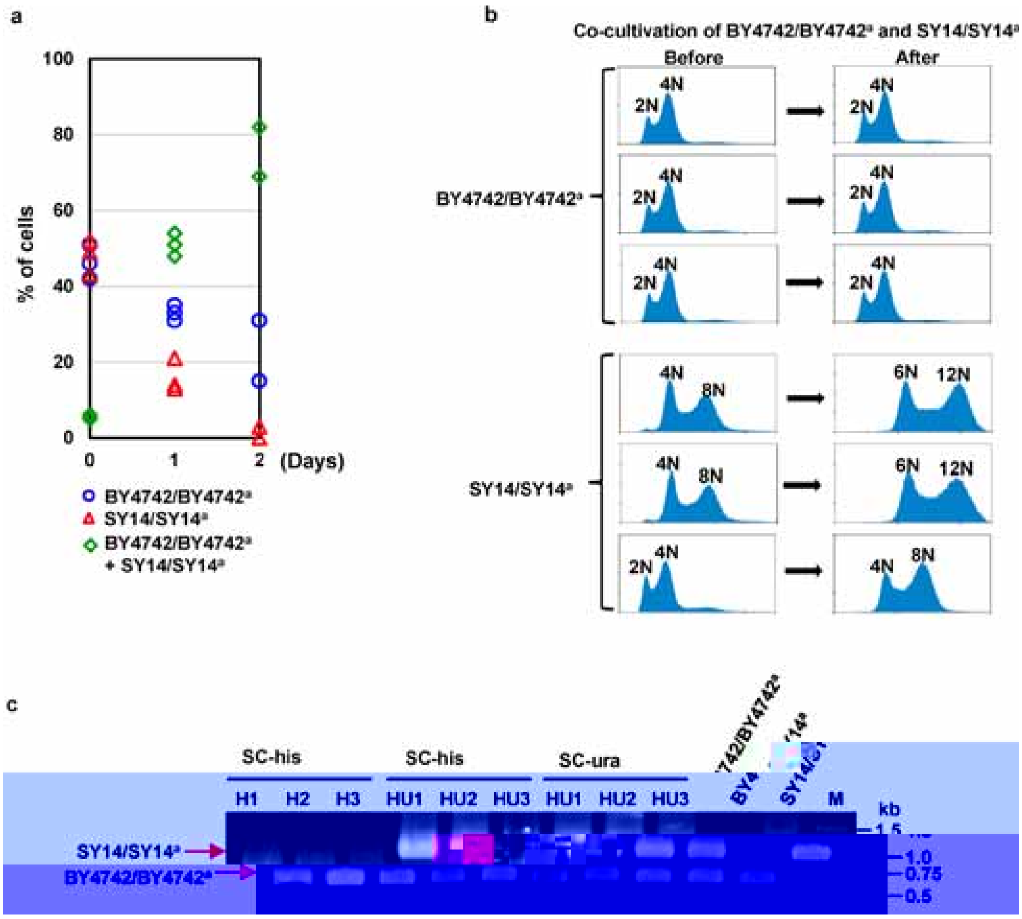
b 0 01 0 01 **d** S S 1 S 1 2 **c** S 2 10 2 S S 1 S 1



Extended Data Fig. 4 3D structures of single chromosomes. S S 1 S 1 2



Extended Data Fig. 5 Directional preference plots of SY6, SY13, and SY14 cells compared to BY4742 cells. 2 *t*
S S 1 S 1 *t* [1]



Extended Data Fig. 6 Growth competition between SY14/SY14a and BY4742/BY4742a diploid cells. a 2 **c** 2 2
2 *S3* S S 1 S 1 1 S
S 1 S 1 *A3* 1 S S 2
S 2 S 1 S 1
2 2 S 1 S 1 S 2 2
S **b** S S 1 S 1
2 2 S 1 S 1

E tended Data Table 1 | Details of the creation of a single chromosome ȷ east

Strain	Chromosomes to fuse (chr. length in kb)	Newly fused chromosome (+) (chr. length in kb)	Newly deleted chromosome regions (RTel: right arm telomere sequence; LTel: left arm tel sequence) (Cen: centromere sequence; RS: repetitive sequence)	No. of transformants for chromosome fusion	Positive rate of chromosome fusion
SY0	VII (1090), VIII (560)	VII+VIII (1609)	VII: 5545-9584 (RS6), 1076068-1090940 (RTel, RS3); VIII: 524501-541100 (RS1), 1-8217 (LTel), 105447-106013 (Cen) V: 18067-23447 (RS11); XV: 10454-13126 (RS9), 21791-30776 (RS10,14);	100	1/3
SY1	XIII (920), XII (1080+1500)	XIII+XII (1980+1500)	XIII: 917281-924431 (RTel, RS7), 268013-268803 (Cen); XII: 1-14474 (LTel)	26	1/3
SY2	I (230), II (810)	I+II (1006)	I: 203183-230218 (RTel, RS1,2); II: 1-9089 (LTel), 237784-238794 (Cen)	11	2/3
SY3	VI (270), XIV (780)	VI+XIV (1036)	VI: 269616-270161 (RTel), 148460-148774 (Cen); XIV: 1-17790 (LTel, RS4)	100	2/3
SY4	XVI (950), V (580)	XVI+V (1505)	XVI: 941976-948066 (RTel); V: 1-8079 (LTel), 151829-152588 (Cen)	143	4/8
SY5	IX (440), X (750)	IX+X (1160)	IX:436361-439888 (RTel), 355607-356006 (Cen); X:1-21750 (LTel, RS9,10,15)	94	1/4
SY6	III (320), IV (1530)	III+IV (1826)	III:313621-316620 (RTel), 114297-114969 (Cen); IV:1-19188 (LTel, RS12)	49	2/4
SY7	XVI-V (1505), VI-XIV (1036)	XVI-V+VI-XIV (2523)	V:569325-576874 (RTel); VI:1-8380 (LTel); XIV:628734-629219 (Cen)	130	1/5
SY8	XV (1090), XI (670)	XV+XI (1725)	XV:1073966-1091291 (RTel, RS8,7); XI: 439551-440264 (Cen), 1-3182 (LTel)	84	3/3
SY9	VII-VIII (1609), IX-X (1160)	VII-VIII+IX-X (2747)	VIII:552000-562643 (RTel); IX:1-11214 (LTel); X:436229-436425 (Cen)	30	2/5
SY10	I-II (1006), III-IV …	I-II+III-IV (2821)	I:151470-1517… II:809896-…	5	2/4

The 'Strain' column lists the strain names, and the number in parentheses indicates the size of the native or fused chromosome in kilobase (kb). An orange plus indicates a fusion event; a dash between two chromosomes means that the fusion already occurred. 'Newly deleted chromosome regions' marks the deleted regions in the corresponding chromosomes; Rtel and Ltel in blue indicate the right arm and left arm of the corresponding telomere sequences, respectively; Cen in red indicates the corresponding centromere sequence; and RS represents repetitive sequences deleted in the corresponding chromosomes. The numbers for each region are referred from the *S. cerevisiae* S288C genome (http://www.yeastgenome.org/).

E tended Data Table 2 | Information regarding long repeat sequences near chromosome ends

Types of repeat sequences	Copy number	Location on chromosomes (bp)	
		RSs to be deleted	Retained RSs
RS1	3	I: 20[illegible]-219229; VIII: 525437-539926	I:13089-27923
RS2	2	I:219230-229411	VIII: 539927-543610 549638- 556001
RS3	2	VII: 1076381-1083886	II: 805133-812631
RS4	2	XIV: 7429-15942	VI: 5531-14039
RS5	2	XI: 658572-665429	III: 4327-11225
RS6	2	VII: 6223-9584	IX: 430983-434367
RS7	3	XIII: 917474-923540; XV: 1078061-1083736	XVI: 7409-13083
RS8	2	XV: 1073988-1078544	XVI: 12601-17099
RS9	3	XV: 11053-13126; X: 8269-10330	IX: 8286-10347
RS10	3	XV:22397-27006; X: 16639-21229	IX:16656-21250
RS11	2	V: 18751-23447	XIV: 772693-777126
RS12	2	IV: 905-18681	X: 727164-744901
RS13	2	XII: 1059296-1064280	III: 303903-308319
RS14	2	XV: 27007-30776	IX: 21251-25254
RS15	2	X:10331-16638	IX: 10348-16655

This Table lists 15 types of long (2 kb) repeat sequences near telomeres, which have two or three copies. Only one copy of each long repeat sequence was retained, and the redundant copies were deleted in the SY14 strain. During the generation of the SY14 strain, six long repeats marked in red (that is, VII: 6223–9584 (RS6), VIII: 525437–539926 (RS1), V: 18751–23447 (RS11), XV: 11053–13126 (RS9), XV: 22397–27006 (RS10), and XV: 27007–30776 (RS14)), which are distal to telomeres, were deleted by two rounds of CRISPR–Cas9-mediated PCR targeting. The remaining 13 long repeats were deleted during chromosomal end-to-end fusions.

E tended Data Table 3 | SNPs and indels confirmed b re-sequencing

a

Ref-Chromosome	Ref-Loci	Ref base	Illumina sequencing		Sanger sequencing		Affected Gene	Variation Loci in Gene	Ref Codon	Variation Codon	Variation Type	Mutation in functional domain? (Y/N)	Null gene phenotypes
			WT	SY14	WT	SY14							
II	9136[#]	G	G	A	G	A	–	–	–	–	–	–	–
III	151645	G	G	A	G	A	–	–	–	–	–	–	–
X	517916	G	G	T	G	T	–	–	–	–	–	–	–
XV	161691	C	C	G	C	G	–	–	–	–	–	–	–
IX	145512	G	G	T	G	T	Nup159	3198	CTC->L	CTA->L	synonymous	–	–
II	447712	A	A	T	A	T	Sif2	4	AGT->S	TGT->C	non-synonymous	N	–
VII	775929	C	C	A	C	A	Skn1	737	GCT->A	GAT->D	non-synonymous	N	–
XV	454309	C	C	A	C	A	Vps5	542	ACA->T	AAA->K	non-synonymous	N	–
X	148553	G	G	A	G	A	Yak1	1838	TCT->S	TTT->F	non-synonymous	Y. Mutation within kinase domain	Null mutant grows slowly, has increased fitness and lifespan, is sensitive to DNA damage.
XI	594128	C	C	A	C	A	Nup133	1304	TCT->S	TAT->Y	non-synonymous	Y. Muta[illegible] within WD40/YVTN repeat-like-containing domain	Null mutant has abnormally elongated bud morphology, decreased cell death, abnormal chemical compound accumulation
XIV	225122	A	A	G	A	G	Sqs1	1978	TGG->W	CGG->R	non-synonymous	Y. Mutation within R3H domain	Null mutant has decreased or increased competitive fitness depend on the growth conditions.

b

Ref-Chromosome	Ref-Loci	Ref base	ALTbase	Ref len	ALT len	Illumina sequencing		Sanger sequencing		Affected	Variation Loci	Mutation in functional	[illegible]
						WT	SY14	WT	SY14				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a, SNPs. **b**, Indels.

E tended Data Table 4 | Differentiall e pressed genes in SY14 compared to BY4742 cells

geneID	GeneSymbol	Expression SY14-1	SY14-2	SY14-3	WT-1	WT-2	WT-3	log_2FC	Pvalue	FDR	Notes
Telomere adjacent genes (<10 kb distance)											
855848	ERR2	0.04	0	0.04	2.08	1.52	2.05	-7.55	7.67E-13	6.78E-11	Chr XVI-L.
855849	HSP32	0	0.1	0	2.86	2.6	2.57	-6.83	2.83E-09	2.03E-07	Chr XVI-L.
855850	FEX2	1.11	4.51	2.73	21.54	21.83	23.7	-3.03	1.64E-12	1.38E-10	Fourth gene near XVI-L.
855852	YPL277C	1.97	2.82	2.09	7.11	5.57	8.61	-1.66	0.000071	0.004003	Sixth gene near XVI-L.
853625	MPH3	3.79	7.58	7.24	19.12	18.21	19.61	-1.65	2.78E-05	0.001664	Second gene near X-R.
854634	VTH1	25.96	23.78	29.85	8.71	8.08	11.03	1.47	0.000126	0.006776	Second gene near IX-L, first gene was deleted in SY14.
851230	SEO1	6.79	7.37	7.27	2.72	2.02	2.69	1.49	0.000254	0.013032	Third gene near I-L, the first two genes were deleted in SY14.
854002	YOL162W	25.86	21.03	21.94	10.17	6.31	5.28	1.64	9.25E-05	0.005119	Fourth gene near XV-L.
854001	YOL163W	42.33	35.02	31.7	14.94	7.6	8.11	1.82	1.53E-05	0.000936	Third gene near XV-L.
850486	THI5	4.61	5.13	4.56	1.51	0.14	1.93	1.93	3.53E-05	0.002072	Fifth gene near VI-L, first gene was deleted in SY14.
853207	MAL11	59.06	56.38	56.36	8.7	9.65	6.49	2.75	1.04E-11	8.32E [illegible]	Fourth gene near VII-R,first three genes were deleted in SY14.
850618	YFR057W	22.58	20.31	18.16	3.13	1.46	0.96	3.40	1.64E-10	1.[illegible]-08	First gene near VI-R.
Stress response											
852364	HSP26	60.43	56.15	60.09	24.96	18.59	20.85	1.44	0.000263	0.013364	Small heat shock protein (sHSP) with chaperone activ
854744	RNR3	15.67	14.26	15.89	6.2	4.95	5.09	1.46	0.000184	0.009714	Minor isoform of large subunit of ribonucleotide-diphosphate reductase;regulated by DNA replication a DNA damage checkpoint pathways. Induced by DNA damage and replication stress.
853326	NCA3	85.44	90.16	83.51	32.42	31	28.38	1.47	0.000148	0.007853	Protein involved in mitochondrion organization; Induc by the SLN1-SKN7 osmotic stress signaling pathway.
850331	HBN1	30.17	22.64	28.11	10.71	6.16	8.97	1.63	9.49E-05	0.005147	Similar to bacterial nitroreductases; protein abundanc increases in response to DN[illegible]ation stress.
855932	OYE3	8.01	5.63	9.95	1.71	2.57	2.83	1.69	7.08E-05	0.004003	Conserved NADPH oxido[illegible]se containing fla mononucleotide (FMN),has potential roles in oxid stress response and programmed cell death.
854944	HUG1	479.95	1084.46	860.2	381.3	251.63	167.35	1.82	0.00048	0.023808	Ribonucleotide reductase inhibitor; transcription induced by genotoxic stress and by activation of Rad53p pathway; protein abundance increases in response to DNA replication stress.
850532	HSP12	211.2	223.37	183.79	62.8	54.47	46.85	1.98	1.56E-06	0.000102	Plasma membrane protein involved in maintainin membrane organization; involved in maintaining organization during stress conditions; induced b shock, oxidative stress, osmostress.; protein abu increased in response to DNA replication stress.
856132	YKU80	12.19	12.28	12.7	0	0.06	0.1	7.65	1.06E-33	1.97E-31	Subunit of telomeric Ku complex (Yku70p-Yku80) involved in telomere length maintenance, structu telo[illegible]osition effect; relocates to sites o strand[illegible]leavage to promote nonhomologous during DSB repair.
Others											
9164931	RDN37-1	82.96	93.16	86.11	13.39	13.71	16.74	2.54	1.53E-10	1.13E-08	35S ribosomal RNA (35S rRNA) transcript.
854565	FIT3	489.5	230.81	526.59	265.15	96.71	154.28	1.25	0.00102	0.049686	Mannoprotein that is incorporated into the c
852298	YBR012W-A	20.41	15.07	15.39	5.28	2.1	5.84	1.91	2.22E-06	0.000143	Retrotransposon TYA Gag gene.
854004	YOL160W	15.64	15.33	26.41	1.1	3.52	1.28	3.23	9.57E-07	6.39E-05	Putative protein of unknown function; conse S. cerevisiae strains.
851850	YDR261W-A	0.24	0	0	1.2	1.94	0	-3.57	3.33E-06	0.000213	Transposable_element_gene.
850849	TAR1	12.98	0	0.08	25.05	66.46	0	-2.88	3.81E-08	2.6E-06	Transcript Antisense to Ribosomal RNA. Pr potentially involved in regulation of respirat metabolism
854860	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Sample size *n* 3. Exact negative binomial two-sided test was used to generate *P* values. Benjamini and Hochberg's algorithm was used to control the FDR.

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