

## **Supporting Information**

# **SCRaMbLEing of a synthetic yeast chromosome with clustered essential genes reveals synthetic lethal interactions**

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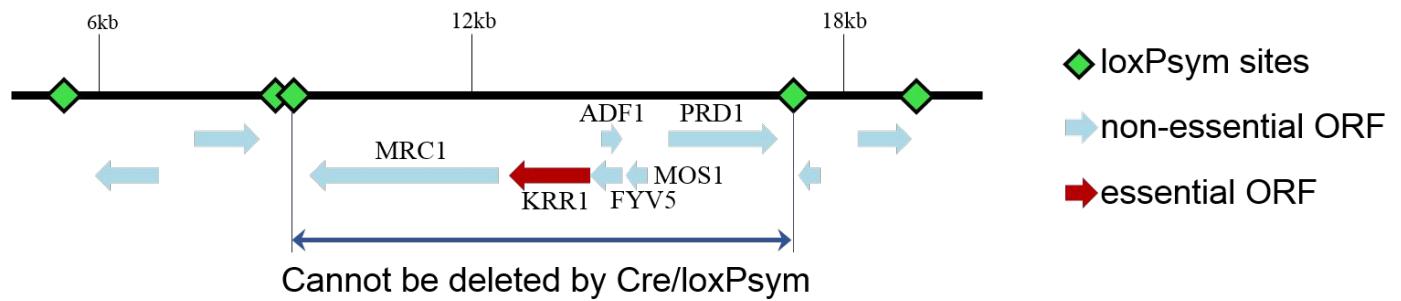
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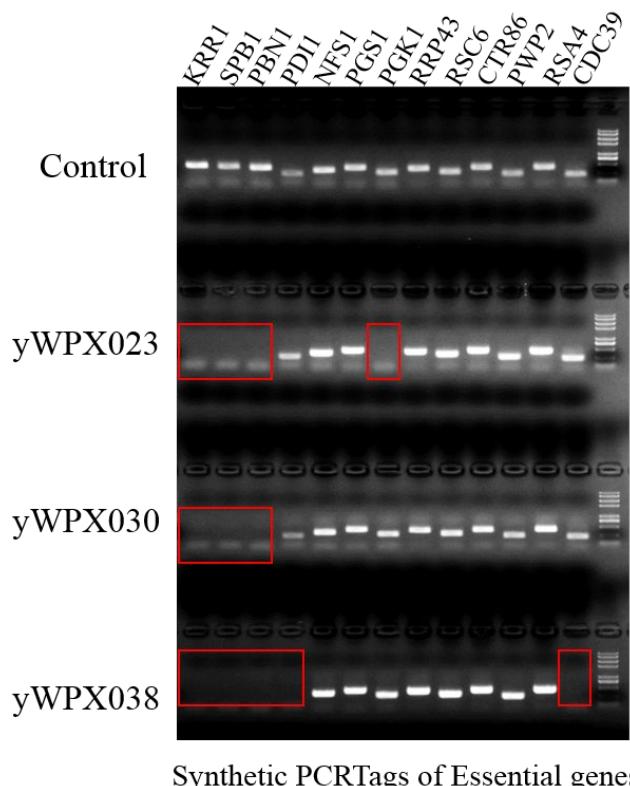
Supplementary Table S5: Primers used in segment knockout and validation

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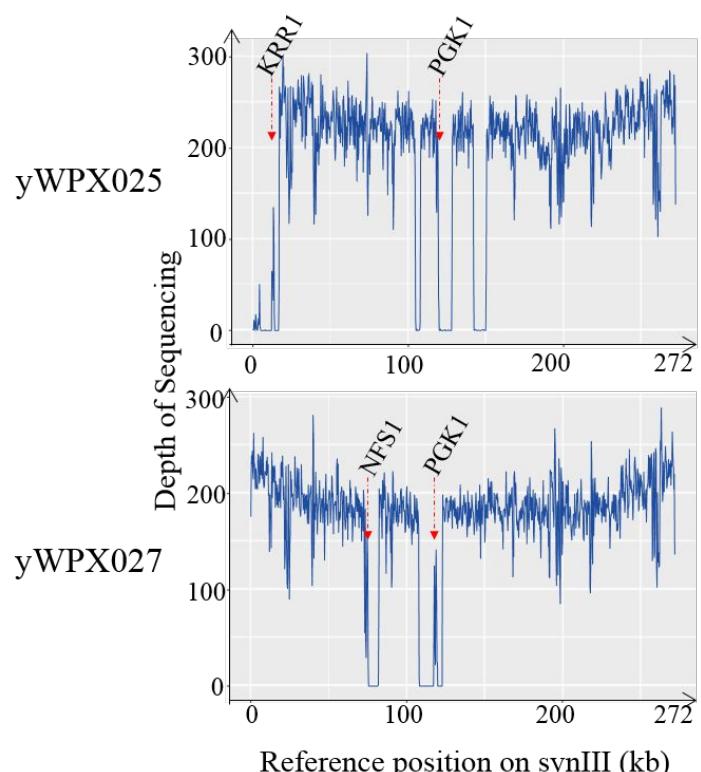


**Supplementary Figure S1.** Schematic diagram of distribution of sectional non-essential and essential genes on synIII. The loxPsym elements were introduced into synIII, which divided the chromosome into sections. Some non-essential genes and essential genes on synIII were located in the same interval.

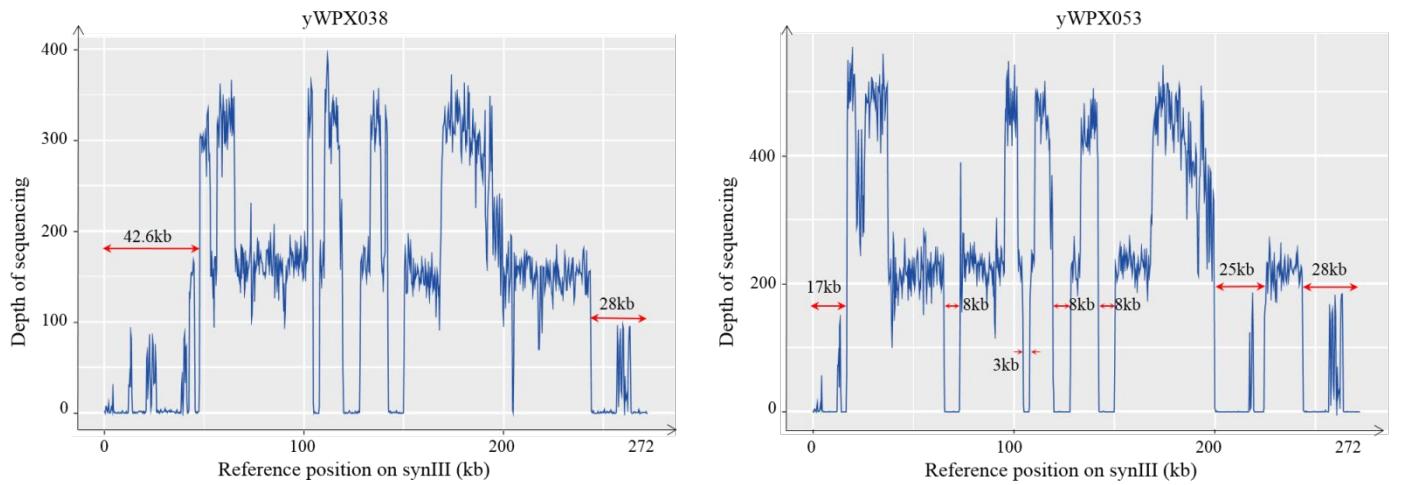
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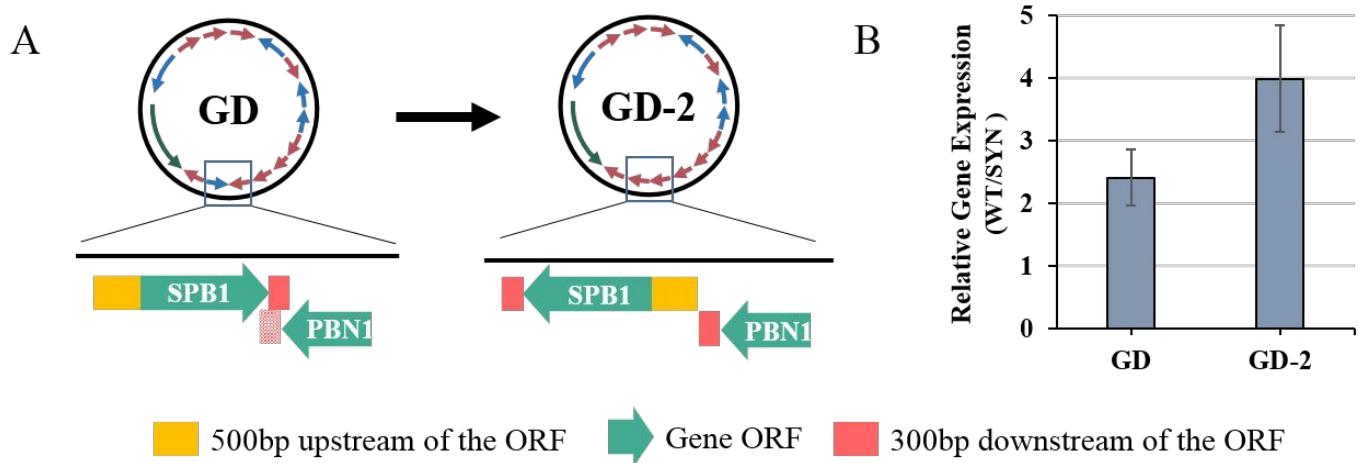
B



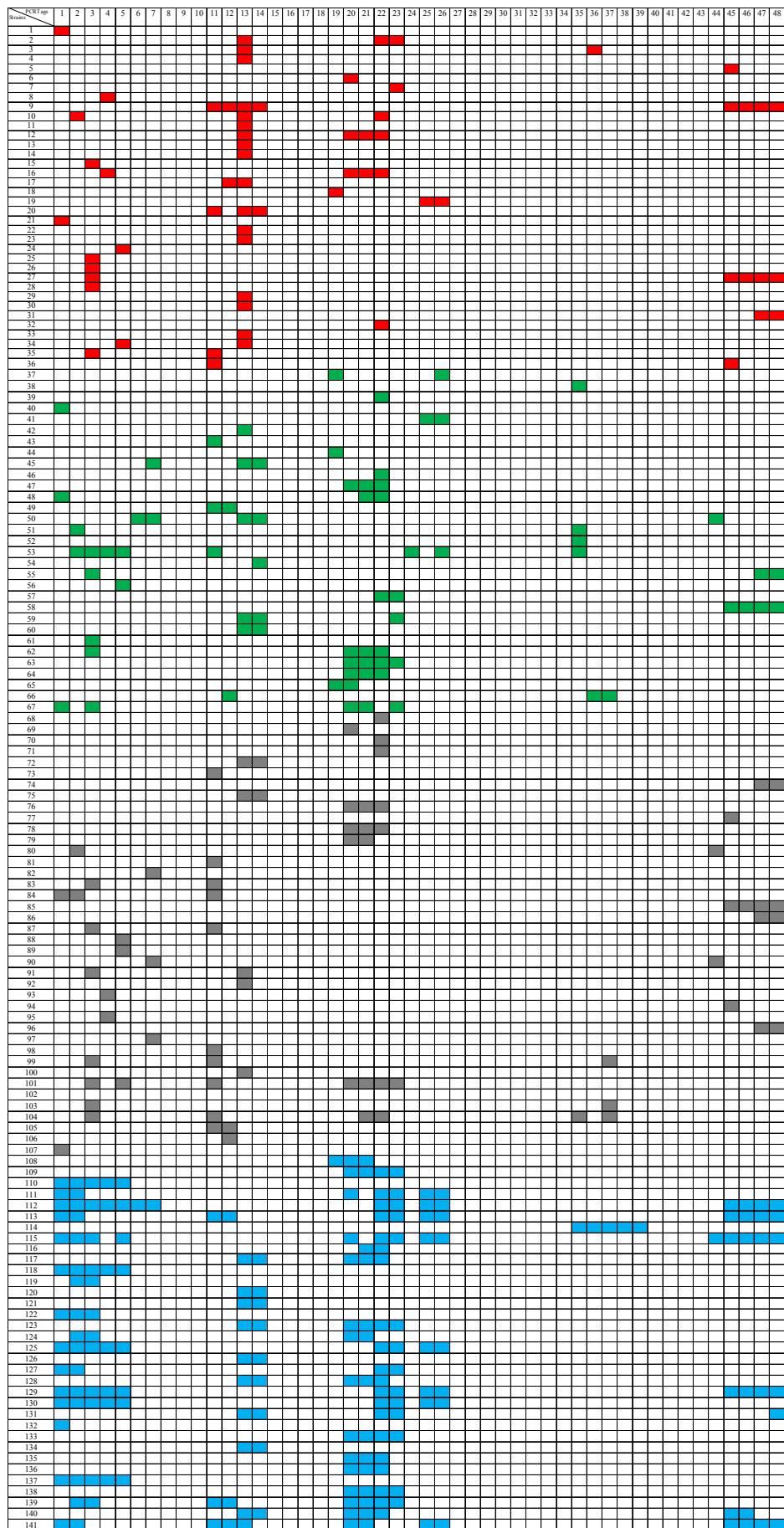
**Supplementary Figure S2.** Schematic diagram of deleting essential genes on synIII by SCRaMbLE with clustered essential genes. (A) Validation of synthetic PCRTags in 13 essential genes. The control strain represents a synthetic yeast harboring synIII. Three of SCRaMbLED strains (yWPX023, yWPX030 and yWPX038) were tested to explain the deletion of essential genes. The essential genes *KRR1*, *SPB1*, *PBN1* and *PGK1* were deleted in yWPX023. The essential genes *KRR1*, *SPB1*, and *PBN1* were deleted in yWPX030. The essential genes *KRR1*, *SPB1*, *PBN1*, *PDI1*, and *CDC39* were deleted in yWPX038. (B) Depth map of synIII from whole-genome sequencing displayed deletion of essential genes. Two of SCRaMbLED strains (yWPX025 and yWPX027) were illustrated to explain the deletion of essential genes. The essential genes *KRR1* and *PGK1* were deleted in a SCRaMbLED strain yWPX025. The essential genes *NFS1* and *PGK1* were deleted in a SCRaMbLED strain yWPX027.



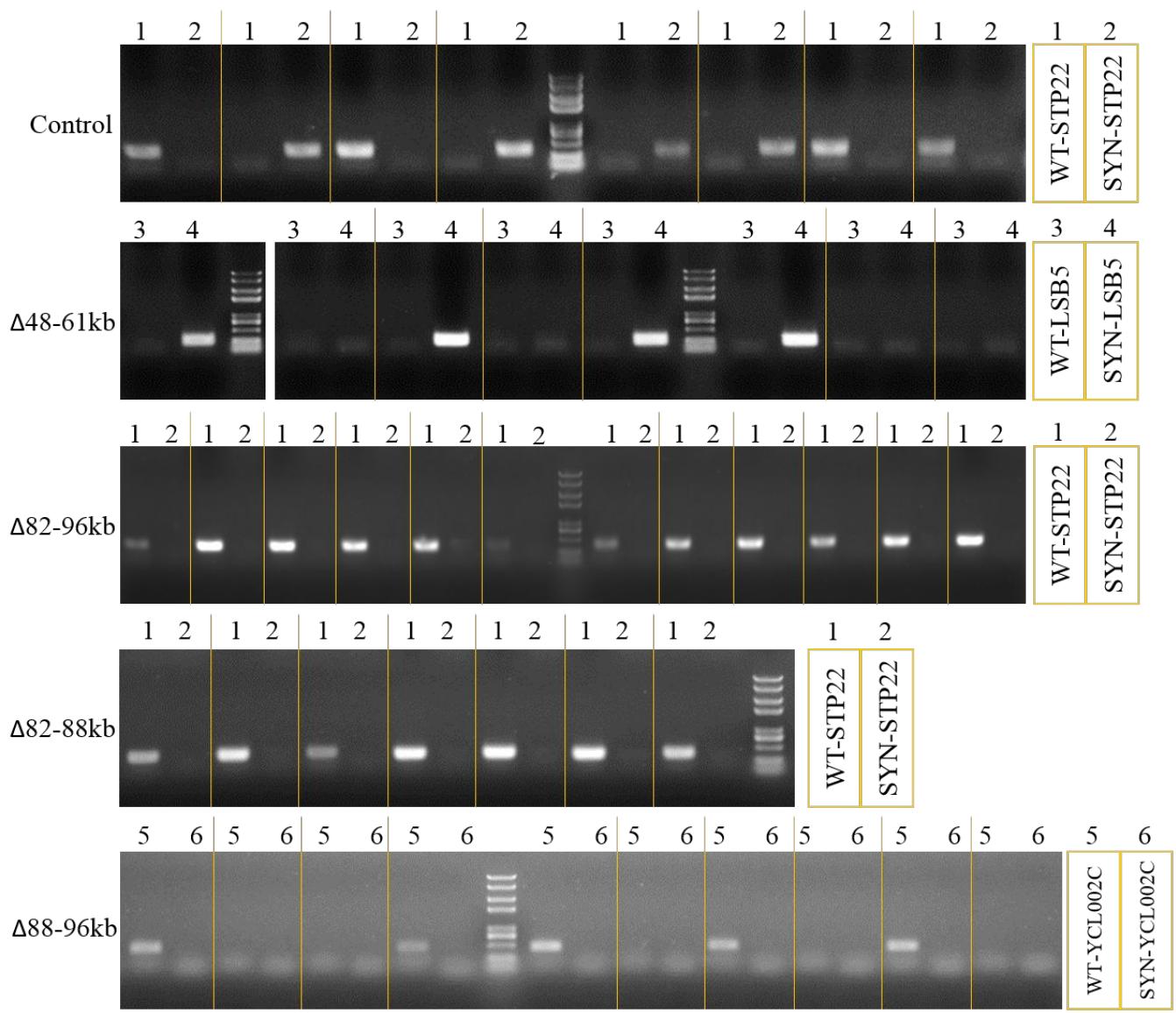
**Supplementary Figure S3.** Genotypes of SCRaMbLEd strains with deletion of large fragments. The genomic sequence comparison between the sequencing strain and the reference strain to show the deletion and replication of the sequencing strain. Due to the presence of clustered essential genes, the depth of essential genes in deleted intervals has not completely disappeared.



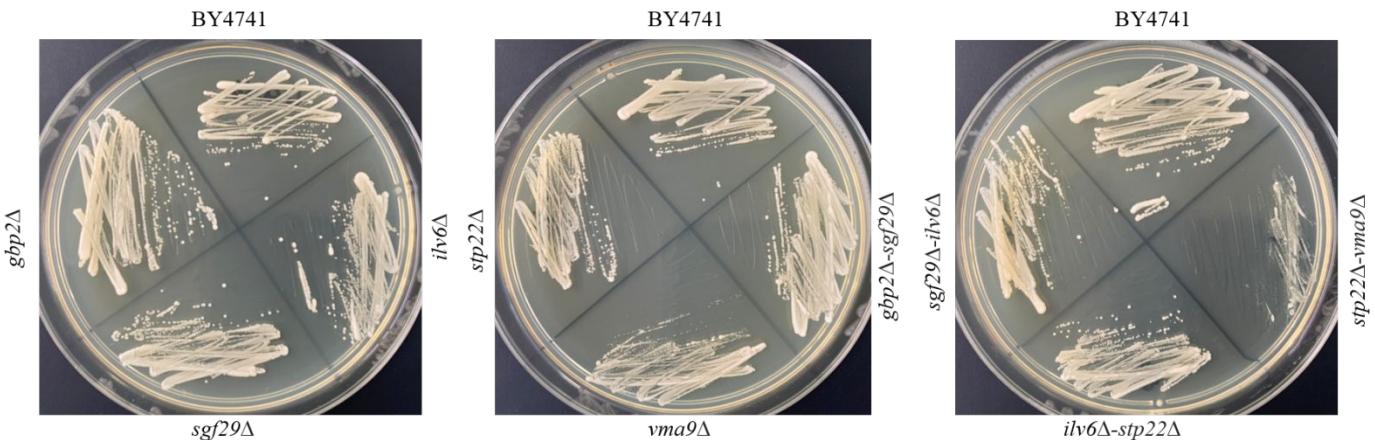
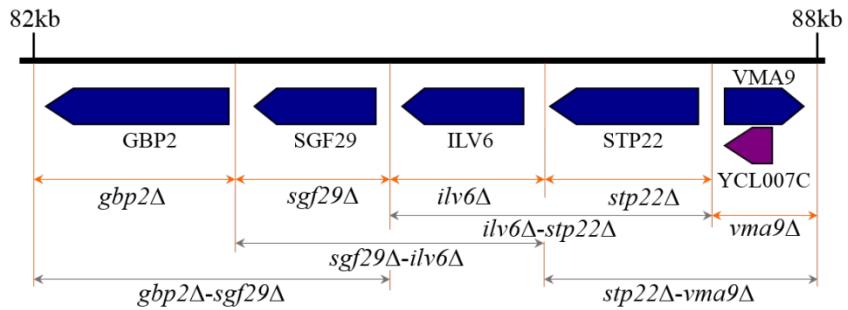
**Supplementary Figure S4.** Real-time qPCR analysis for the validation of gene expression. (A) Schematic diagram of gene order in GD plasmid and inversion of *SPB1* in GD-2 plasmid. The yellow, green and red bars represent 500bp upstream the gene ORF, gene ORF, and 300bp downstream the gene ORF, respectively. (B) The bar chart shows relative gene expression of *SPB1*.



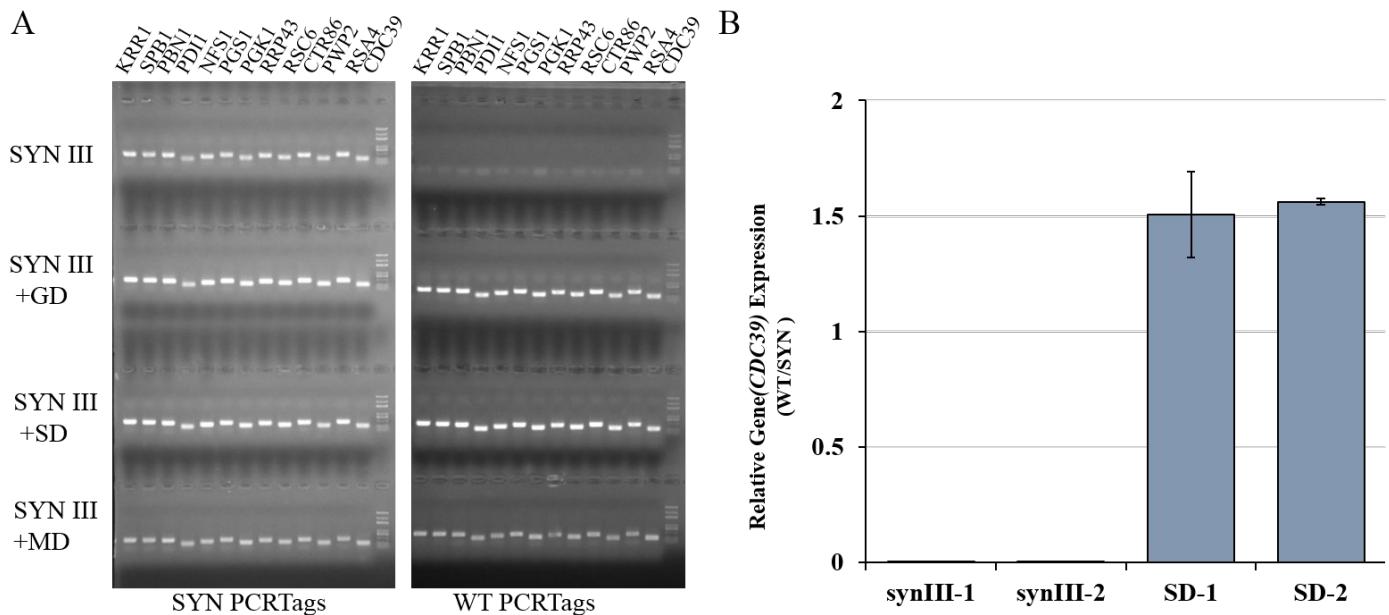
**Supplementary Figure S5.** Deletions of the 141 SCRaMbLEd strains in 48 pairs of PCRTags. The white bars represent the parental types. The red, green and grey bars represent deletions in SCRaMbLEd strains that were SCRaMbLEd only once and harbored GD, SD and RD plasmids, respectively. The blue bars represent the deletions in strains with multiple rounds of SCRaMbLE.



**Supplementary Figure S6.** PCRTag analysis of spores. The control group refers to spores produced from a diploid strain that was obtained by mating BY4741 with synIII. Spores derived from diploid strains with individual deletion of synIII 48-61kb, 82-96kb, 82-88kb, and 88-96kb were tested. From control, Δ48-61kb and Δ88-96kb groups, complete four spores could derived. Haploid spores with deletion of synIII 82-96kb or 82-88kb were inviable.



**Supplementary Figure S7.** Phenotype of strains with the individual ORFs and adjacent two ORFs knocked out in the region of synIII 82-88kb. The strains were grown on YPD medium at 30°C.



**Supplementary Figure S8.** Validation of the assembly of SD plasmid. (A) Verification of wild-type (WT) and synthetic (SYN) essential genes on three plasmids using PCRTags. (B) QPCR analysis of the transcription of *CDC39* in the correct SD plasmid. The bar chart shows relative gene expression of *CDC39*.

**Supplementary Table S1.** PCRTags of synthetic chromosome III used in this study

PCR Tags	ORF	Forward primer (5'-3')	Reverse primer (5'-3')
1	<i>YCL064C</i>	ATCTTGATGGTGTGCTGCTACCAACCG	CAGCTGGTACCGCTGTTATTAGCAAC
2	<i>YCL059C</i>	GTCGACTTTCGTGGTAGTTGAGCTGGT	GCTAGCTGAAGCGTCCCATTCCA
3	<i>YCL051W</i>	TGACGCTCTAAAACCCGACCACGAAGC	CTTGCTTGGAGTGTAAAGCAAGCTGCTT
4	<i>YCL049C</i>	GACGTGATAATATCGGCTCCAGGCAGAG	AATTAGCGTCTCTACAACCTGGACCAGC
5	<i>YCL045C</i>	TAGTTGGCTGTCGCTACCAGGTAGCAAG	TCCTGACGAACGACTAACGAATAGCTCT
6	<i>YCL043C</i>	GAAAGCTTCTCGCTCAATTGTGGTAGG	TGAATCTGGCCTACCACCTAGGGTATTG
7	<i>YCL039W</i>	CTCTAGCCCATTGCGAAGCGAATGGTTG	GCTAGCGCTGTTGGTAACAGGGTAAAAA
8	<i>YCL033C</i>	ATCTAGCGTTCTCGACCAAGCAGCAGC	GCTCAAAGCGCTTGGAAATGAGTGACA
9	<i>YCL030C</i>	GTAGACACCTCGGCCCTAGGTCAATAGCT	AAGCCTACCTCTGGCAAGTTTCTGAC
10	<i>YCL029C</i>	GGCTTTCGATCGCTGCTCAAAATGCTA	TAGCATCAGCCAAACTAGCCGACGAACC
11	<i>YCL026C-A</i>	TAGGTTGGCACCTAGACCTAGCAATTCC	CGCCGAAGCTAAAAAACGACCTGAAAGC
12	<i>YCL025C</i>	GCCTAGTTACCCCAAGCTCGACCTTGA	TGCTGTCATCTTGTGAGCGTTTGAGC
13	<i>YCL017C</i>	GCCGCTGCTCAAAGCAATGTCTCGAAA	AGGTATCGCGCTATTACGTTGACGA
14	<i>YCL014W</i>	GATCAGCCAACGACACCCAAAAGCCCT	GCTGCTGCTGTTGGTAGCTTACCGATG
15	<i>YCL011C</i>	AACGCTGCCAAAACCTCGGCTGAAACCA	CGCTATCAGCAAATTGACGGCGTTTG
16	<i>YCL008C</i>	AACGTGGCTGGTGTAGAGAATTGTTGA	AAGCCCTCATTGAAGCCACCTCTACCA
17	<i>YCL002C</i>	AGCGTTAGTTGGATAACCAATCGGCC	CAGCAGCTGGTTCGAGAACATTGAGC
18	<i>YCR002C</i>	AGCATTAGAGCTATGTGGCGCTGCTT	TTTCGAGGTCGAAAGACCCGATGGTCT
19	<i>YCR005C</i>	ACCTGGAGCAACTTCATAGATGCTGCTG	TAGCGCTTGTAGCAGGCCATACTTGAGC
20	<i>YCR009C</i>	AGATAGTTCTTTCGGCTCGAGGCAAC	TCTAGATAGCCTACGAGCCGTTACCGCT
21	<i>YCR012W</i>	TCTAGCCAGCCATCTAGGCCGACCTAAT	GTCGGCTAGGCTAGATAGTTATGTCGA
22	<i>YCR015C</i>	AACTTGTGCGTACCGGTTAGCAATCGG	AAGCTGCCACTATTGAGCAGCGGTGTT
23	<i>YCR017C</i>	CAATTACCCGACAGGGCTTGGTAGCAAG	TTTGTGAGCTTGACCGCCGATTGTT
24	<i>YCR023C</i>	GCTGTTGGCTTGGCTGTATAAGCG	CAGCTCTCCACTATCTGCCAAGCGTT
25	<i>YCR026C</i>	CAAGCTGGGTGAAAGCCATCCAAGCTG	TGCTAAGGACTCTCGAGACGGCAGCAAT
26	<i>YCR027C</i>	GGCCAACCTTTCACCTTCGGCCTGGTA	GAGCCTAACCGGTGTTAGAGGTATCATC
27	<i>YCR030C</i>	TGGAGGGCTAGATTGTTAGGGGTGCTT	ATTGGCTAGCGCTAGCAGCTTTGACC
28	<i>YCR032W</i>	GGCTGGTCGAAGCTTAATGACCTAATC	GCTAGACCAAGCTCGTCCAAGCTAGAG
29	<i>YCR033W</i>	ATCTAGCGTTCTCGACCAAGCAGCAGC	GCTCAAAGCGCTTGGAAATGAGTGACA
30	<i>YCR034W</i>	GCCTTGTCTACCCCTACCACCAAGTTTG	AACCCAGCTATGCTGGTAGTACCCATT
31	<i>YCR038C</i>	AGGGTGAACAACAACCTCAATCGTTGG	TGCCCTGAATGTTAGCCCTGGAGCTTG
32	<i>YCR045C</i>	TCGTTTAGTCTGATTCGCCAATGGC	TGGTGTGAAATCGAAAGCTTGAGCCAC
33	<i>YCR052W</i>	CCGACGACGATTGGACACCAGCATTAAAC	CAAAGATAGGCTGCTGTCTCGCTTTCGT
34	<i>YCR054C</i>	ATCCTTTGTGGGTGTCGTTCTCGA	ACCTGCTAGCTGAACGAAAACCTAGC
35	<i>YCR061W</i>	CGGTACTACCAGCGTTGTTGGTC	GCTGCTCAAGCTAAATAGGGTATCTCGA
36	<i>YCR065W</i>	GAGCCCCAGACCATAGCAGCCCT	GCCTTGGCGTCACAGCTTTTCGGTT
37	<i>YCR067C</i>	GATGCTATCTGGCTAGCGCTGGTAGCTT	AAGCTTGAGCCATATGCCAAGCAGCAGC
38	<i>YCR072C</i>	GCAGCTAACCAACAATCTACAATCGCTG	CCTAAAAAGCCACGCTCATTGGGTCAAC
39	<i>YCR073C</i>	AACACCGCTTGATGTAGGTAAGCTAGG	TTTGTGAGCTTGGCTAGCAGCTTGAGC
40	<i>YCR075C</i>	GCTAGCCAAGTCGTGACCTCTGCTT	GGCTAGCTGGCTATCTCAGCTTGTGTT
41	<i>YCR077C</i>	GCTGCTGCTACCGCTGCTAGCT	CCGAGCTTACCTAGAACACAGCGGTGAT
42	<i>YCR081W</i>	ACACGAACATCTAGCAGCCATACCAGC	CAACAAATGCATGCTGATAGCGTCGTCA
43	<i>YCR084C</i>	GCTGTCCTAGCTAGCAAAATGGAGGA	CGGCAGCCCTAGCGCTTTCC
44	<i>YCR088W</i>	TGCTCTTCTTGGCAAGCCGAAATAGC	GTTAGATGGAAACAAGCCCTGCTACCA
45	<i>YCR089W</i>	CAGCTCTAGCAGCTGGCTAGCACTAAG	GCTGCTTGGCAAGCTATAACCGCTGCTA
46	<i>YCR091W</i>	TGAAGCTAAGCATTGGGCAGCAAGAGC	AGATCGGTGGCTAGAATTGTCGAGGTT
47	<i>YCR093W</i>	TTTCGGTGGTGAAGGCAGCATCAGCCAT	AGCGGCGGTTTTAGTTGCTTCGTCG
48	<i>YCR095C</i>	GCTGTGGTTCGGTTGTTCCAATGGT	TGCTGTCCCAGTTAAGCTAGCAGCGTT

**Supplementary Table S2.** Whole genome sequencing strains involved in this study

Strains	Sample name	File name 1	File name 2
1	yWPX001	yWPX001.IS300_Clean.1.fq.gz	yWPX001.IS300_Clean.2.fq.gz
2	yWPX019	yWPX019.IS300_Clean.1.fq.gz	yWPX019.IS300_Clean.2.fq.gz
3	yWPX020	yWPX020.IS300_Clean.1.fq.gz	yWPX020.IS300_Clean.2.fq.gz
4	yWPX021	yWPX021.IS300_Clean.1.fq.gz	yWPX021.IS300_Clean.2.fq.gz
5	yWPX023	yWPX023.IS300_Clean.1.fq.gz	yWPX023.IS300_Clean.2.fq.gz
6	yWPX025	yWPX025.IS300_Clean.1.fq.gz	yWPX025.IS300_Clean.2.fq.gz
7	yWPX027	yWPX027.IS300_Clean.1.fq.gz	yWPX027.IS300_Clean.2.fq.gz
8	yWPX028	yWPX028.IS300_Clean.1.fq.gz	yWPX028.IS300_Clean.2.fq.gz
9	yWPX029	AT08406.IS300_Clean.1.fq.gz	AT08406.IS300_Clean.2.fq.gz
10	yWPX030	AT08407.IS300_Clean.1.fq.gz	AT08407.IS300_Clean.2.fq.gz
11	yWPX031	yWPX031.IS300_Clean.1.fq.gz	yWPX031.IS300_Clean.2.fq.gz
12	yWPX032	AT08408.IS300_Clean.1.fq.gz	AT08408.IS300_Clean.2.fq.gz
13	yWPX033	AT08409.IS300_Clean.1.fq.gz	AT08409.IS300_Clean.2.fq.gz
14	yWPX034	AT08410.IS300_Clean.1.fq.gz	AT08410.IS300_Clean.2.fq.gz
15	yWPX035	AT08411.IS300_Clean.1.fq.gz	AT08411.IS300_Clean.2.fq.gz
16	yWPX036	AT08412.IS300_Clean.1.fq.gz	AT08412.IS300_Clean.2.fq.gz
17	yWPX037	yWPX037.IS300_Clean.1.fq.gz	yWPX037.IS300_Clean.2.fq.gz
18	yWPX038	yWPX038.IS300_Clean.1.fq.gz	yWPX038.IS300_Clean.2.fq.gz
19	yWPX039	yWPX039.IS300_Clean.1.fq.gz	yWPX039.IS300_Clean.2.fq.gz
20	yWPX040	yWPX040.IS300_Clean.1.fq.gz	yWPX040.IS300_Clean.2.fq.gz
21	yWPX041	yWPX041.IS300_Clean.1.fq.gz	yWPX041.IS300_Clean.2.fq.gz
22	yWPX050	yWPX050.IS300_Clean.1.fq.gz	yWPX050.IS300_Clean.2.fq.gz
23	yWPX052	AT08420.IS300_Clean.1.fq.gz	AT08420.IS300_Clean.2.fq.gz
24	yWPX053	yWPX053.IS300_Clean.1.fq.gz	yWPX053.IS300_Clean.2.fq.gz
25	yWPX062	yWPX062.IS300_Clean.1.fq.gz	yWPX062.IS300_Clean.2.fq.gz
26	yWPX098	AT08421.IS300_Clean.1.fq.gz	AT08421.IS300_Clean.2.fq.gz
27	yWPX099	AT08422.IS300_Clean.1.fq.gz	AT08422.IS300_Clean.2.fq.gz
28	yWPX101	AT08388.IS300_Clean.1.fq.gz	AT08388.IS300_Clean.2.fq.gz
29	yWPX103	AT08390.IS300_Clean.1.fq.gz	AT08390.IS300_Clean.2.fq.gz
30	yWPX107	AT08394.IS300_Clean.1.fq.gz	AT08394.IS300_Clean.2.fq.gz
31	yWPX108	AT08395.IS300_Clean.1.fq.gz	AT08395.IS300_Clean.2.fq.gz
32	yWPX110	AT08397.IS300_Clean.1.fq.gz	AT08397.IS300_Clean.2.fq.gz
33	yWPX111	AT08398.IS300_Clean.1.fq.gz	AT08398.IS300_Clean.2.fq.gz
34	yWPX112	AT08399.IS300_Clean.1.fq.gz	AT08399.IS300_Clean.2.fq.gz
35	yWPX114	AT08401.IS300_Clean.1.fq.gz	AT08401.IS300_Clean.2.fq.gz

**Supplementary Table S3.** Yeast strains and plasmids used in this study

Designation	Description	Remarks
strains		
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	
yLM197	<i>MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SYN3-272123bp SUP61(tRNA)</i> integrated at HO	Narayana <i>et al.</i> [11]
plasmids		
pLM006	pRS413-SCW11-Cre-EBD	Narayana <i>et al.</i> [11]
GD	pRS413, KRR1-SPB1-PBN1-PDI1-RRP7-NFS1-PGS1- PGK1-RSC6-CTR86-RRP43-PEP2-RSA4-CDC39	This study
SD	pRS413, KRR1-SPB1-PBN1-PDI1-RRP7-NFS1-PGS1- PGK1-RSC6-CTR86-RRP43-PEP2-RSA4-CDC39	This study
RD	pRS415, PDI1-KRR1-SPB1-PBN1-RSC6-RSA1-PGS1- PGK1-NFS1-RRP7-RRP43-PWP2-CTR86-CDC39	This study

**Supplementary Table S4.** Primers used in this study for quantitative PCR (qPCR)

Primers	Forward primer (5'-3')	Reverse primer (5'-3')
WT-CDC39-1	TCTTTCTCTTCACCTGCAGAGGAGCTT	CGACTGGGATGGCTGATTCAAACGGCTT
SYN-CDC39-1	CTTGAGCAGCAGCCCAGCTGAAGAATTG	GCTTTGGCTAGGTTGGTTAGTCGAGAC
WT-SPB1-1	TAGAAGGTCGGAAAGGGATGCCAAGTTT	GGAGTCATCACTGAACCTCTCATCCGAA
SYN-SPB1-1	CCGACGAAGCGAACGAGACGCTAAATTC	GCTATCGTCAGAGCTAGATTGTCGCTG
WT-SPB1-2	TGCATCCAAACTCTGTCTGTCAACTCC	TAATCTTGGTCCAGCCTTTGGTGCC
SYN-SPB1-1	CGCTAGCAAGTTGTGCCAGTTAATAGC	CAATCGAGGATCCAATCGTTAGGAGCT
WT-SPB1-3	GGCACATCAGTTAGCATTGGTCAGAAA	GTCTGAATCATCGTTAATCAAGCCGCC
SYN-SPB1-1	AGCTCACCAATTGGCTCTAGGCCAAAAG	ATCGCTGTCGTATTGATTAGACCAGCT

**Supplementary Table S5.** Primers used in segment knockout and validation

Regions/primers	Forward primer (5'-3')	Reverse primer (5'-3')
synIII 48-61kb	taagcgtagccatcccttgatattg	TAAAAAAAAAATGATGAATTGAATTGAAAAA GCTGTGGTATtgaatccggatcttcctataaagg
synIII 48-61kb	ACAAATTAGAGCTCAATTAAATTATATC AGTTATTACCCatgattcagttcgcccttatcc	tagtatacaggaatgcaccaac
synIII 82-96kb	agaacatgcgcgtgagaattatcg	TAAAAAAAAAATGATGAATTGAATTGAAAAA GCTGTGGTATtggaccctattgtatccaaagc
synIII 82-96kb	ACAAATTAGAGCTCAATTAAATTATATC AGTTATTACCCtaatagcttcgcaggtagc	agaacggatacaacacccgatc
synIII 82-88kb	agaacatgcgcgtgagaattatcg	TAAAAAAAAAATGATGAATTGAATTGAAAAA GCTGTGGTATtggaccctattgtatccaaagc
synIII 82-88kb	GCTCAATTAAATTATATCAGTTATTACCCt gtcgaggatatacggcaag	tcctctacagttgctgatg
synIII 88-96kb	tggtagagggtggctcaatgg	TAAAAAAAAAATGATGAATTGAATTGAAAAA GCTGTGGTATacgtcacccgttgcataactcg
synIII 88-96kb	ACAAATTAGAGCTCAATTAAATTATATC AGTTATTACCCtaatagcttcgcaggtagc	agaacggatacaacacccgatc
synIII 151-200kb	agagctatcacaggcatttac	TAAAAAAAAAATGATGAATTGAATTGAAAAA GCTGTGGTATgtaccaaatagctaactgc
synIII 151-200kb	ACAAATTAGAGCTCAATTAAATTATATC AGTTATTACCCacgaagtattgattacgcggatcgaa	tcatgctttaccgttgctc
gbp2	cataaaagtacacaggcatggcggtggtagaaGAGTG CACCATAACCACAGC	gaaaaaggaaacaaacatcagctggatccccgcacAGT TTTTAGTTTGCTGGC
sgf29	agaagatctttagatatgttagtaatgttaaccaccattgGAGTGC ACCATAACCACAGC	ggatccatcagcaaaaacacccgttgcacccatttACAGTT TTTAGTTTGCTGGC
ilv6	aggagagtcccggggcgatcgcaaggccggagagactaacGA GTGCACCATACCACAGC	aatctttagaaacatctgatctactaaccggatctttaACAGTT TTTAGTTTGCTGGC
stp22	atattttatggacttcggcgatgcgaaagaaagttagGAGTG CACCATAACCACAGC	atcttaacggccaagaaaagagagatgttagaaacggACAG TTTTAGTTTGCTGGC
vma9	aagaacaaaggccaacgaaataaaagaacaaagactaGAG TGCACCATACCACAGC	atactcaggaaacgtcacccgttgcataactcgacgtACAGTT TTTAGTTTGCTGGC
gbp2- sgf29	cataaaagtacacaggcatggcggtggtagaaGAGTG CACCATAACCACAGC	ggatccatcagcaaaaacacccgttgcacccatttACAGTT TTTAGTTTGCTGGC
sgf29-ilv6	agaagatctttagatatgttagtaatgttaaccaccattgGAGTGC ACCATAACCACAGC	aatctttagaaacatctgatctactaaccggatctttaACAGTT TTTAGTTTGCTGGC
ilv6-stp22	aggagagtcccggggcgatcgcaaggccggagagactaacGA GTGCACCATACCACAGC	atcttaacggccaagaaaagagagatgttagaaacggACAG TTTTAGTTTGCTGGC
stp22-vma9	atattttatggacttcggcgatgcgaaagaaagttagGAGTG CACCATAACCACAGC	atactcaggaaacgtcacccgttgcataactcgacgtACAGTT TTTAGTTTGCTGGC
V-GBP2	tgcctttcgacatctatcg	tgtccacattgtatgaaactcgac
V-SGF29	agtgaataacatcacttcgcacg	agtggcagatcgactggac
V-ILV6	tacacctgtacaaacgtacaatc	tctacgcgcgtcttagactatacc
V-STP22	taccattcccggtcttgtttagg	tgacgagacagaccgtcatg
V-VMA9	tggccgttaagatccaactg	acttctccaaacactgtacgac
V-URA	tgcctttcgacatctatcg	TGCCTTAGCGGCTTAAC