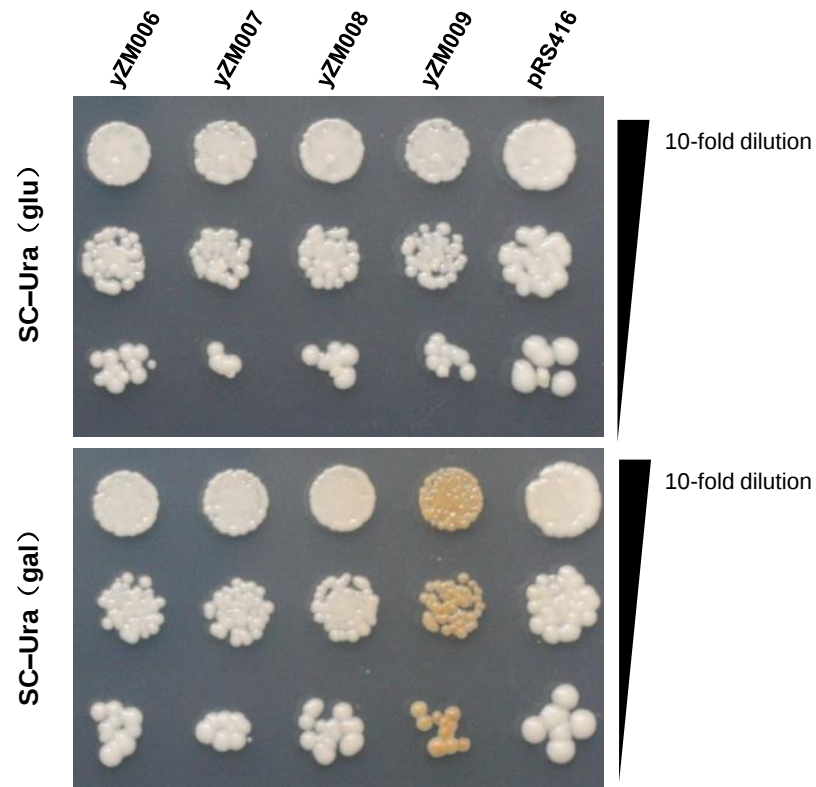
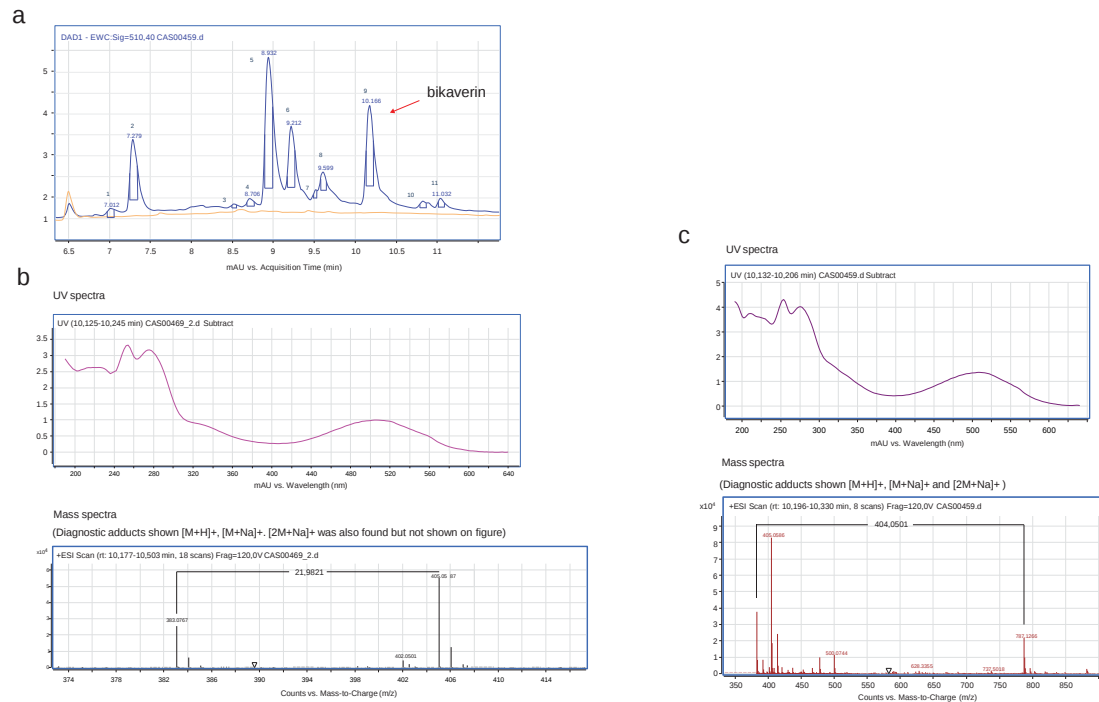


**Pathway engineering in yeast for synthesizing the complex polyketide
bikaverin**

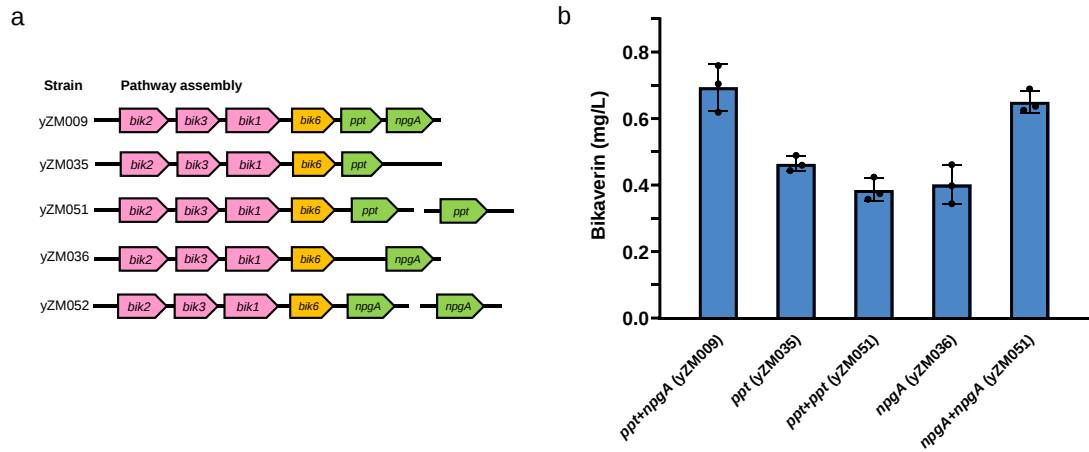
Zhao et al.



Supplementary Fig. 1. Phenotypes of bikaverin pathways with *bik1* driven by different promoters, growing on SC-Ura plate with glucose or galactose as carbon source for 2 days. yZM006, *P_{RPS2}-bik1*; yZM007, *P_{RPL43A}-bik1*; yZM008, *P_{GPM1}-bik1*; yZM009, *P_{GAL1}-bik1*; pRS416, empty control.



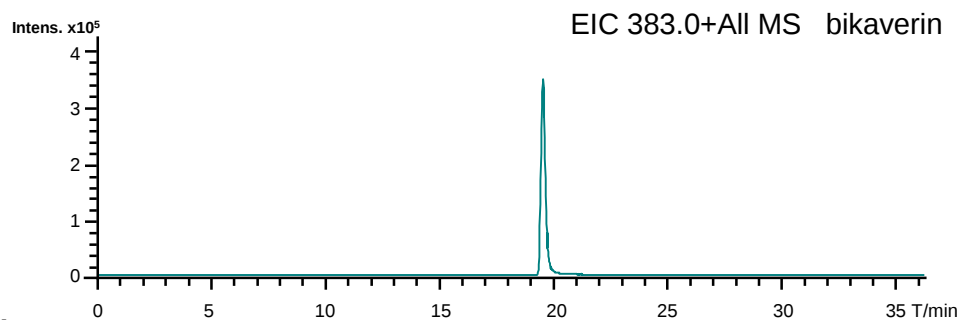
Supplementary Fig. 2. Bikaverin analysis in yZM009 by HPLC, UV spectra and Mass spectra. Strain yZM009 grown in SC–Ura medium with galactose as the carbon source was analyzed by HPLC at 510nm as shown in **a**. (Blue line: yZM009; yellow line: empty control). Red arrow indicated the peak of bikaverin. UV spectra and Mass spectra of bikaverin standard were shown in **b**. The UV spectra and Mass spectra for bikaverin detection in yZM009 were shown in **c**. The UV spectra and Mass spectra were extracted from the peak 9 as shown in **a**.



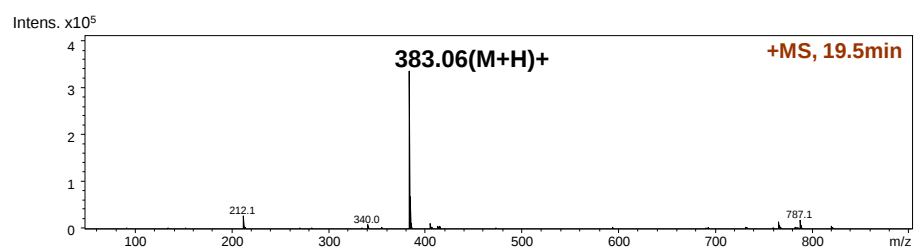
Supplementary Fig. 3. Results from shake-flask fermentation with different strains carrying different copies of Pptase. Genotypes of strains were shown in **a** and titers of bikaverin were shown in **b**. Data are presented as mean values $\pm$ SD and error bars show SD from $n=3$ biological replicates. Source data underlying Supplementary Figure 3b are provided as a Source Data file.

HPLC/ESI-MS analytical standard

a

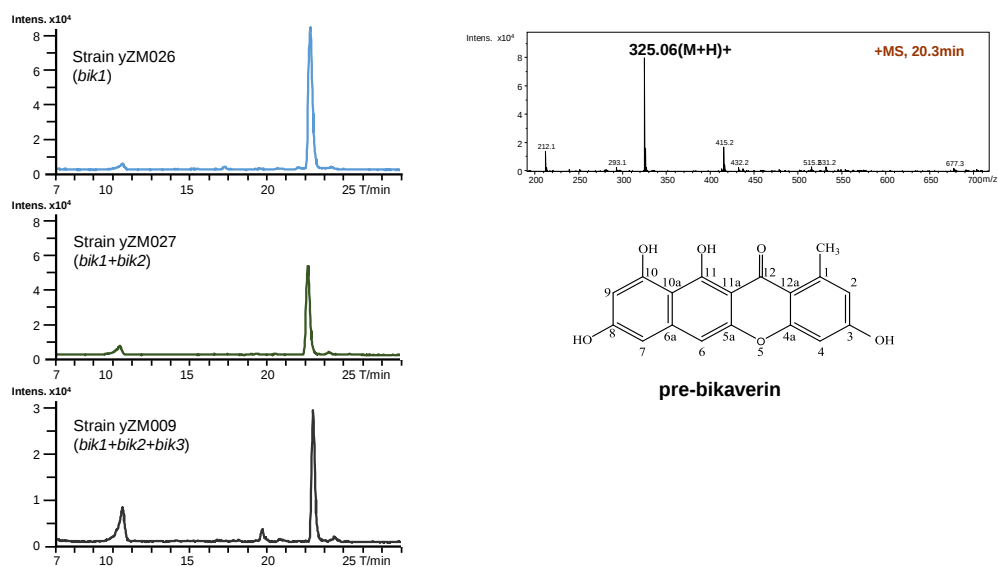


b

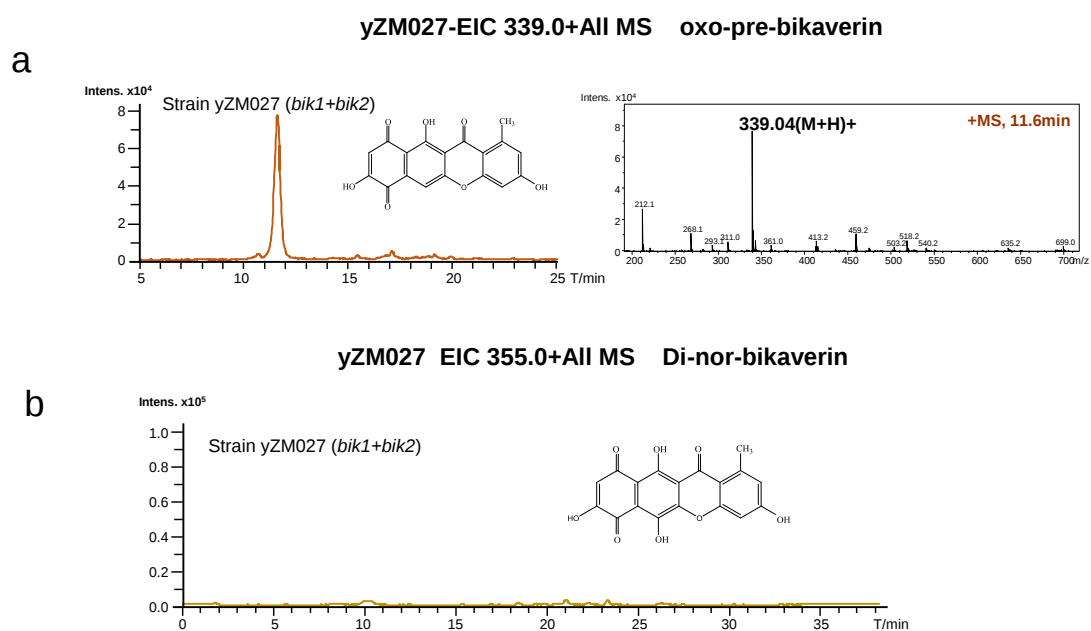


Supplementary Fig. 4. The Bikaverin standard for HPLC/ESI-MS analysis. **a.** the extracted ion chromatogram (EIC) of m/z 383.1, the mass of $[M-H]^+$ of bikaverin. **b.** the mass spectrum of the bikaverin peak in **a.**

EIC 325.0+All MS Pre-bikaverin

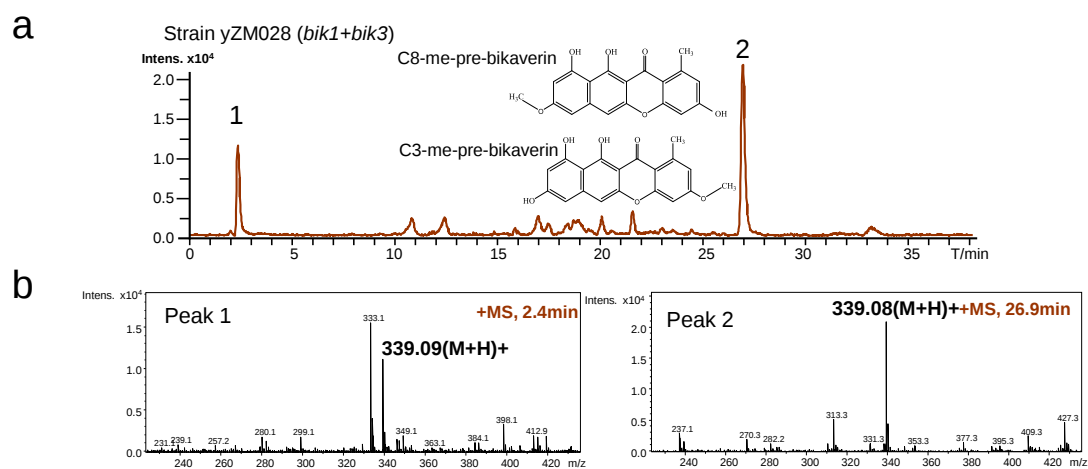


Supplementary Fig. 5. The EIC of pre-bikaverin with m/z 325.1 in strains yZM026, 027 and 009. The mass spectrum of the corresponding peak in yZM026 is shown on the right side.

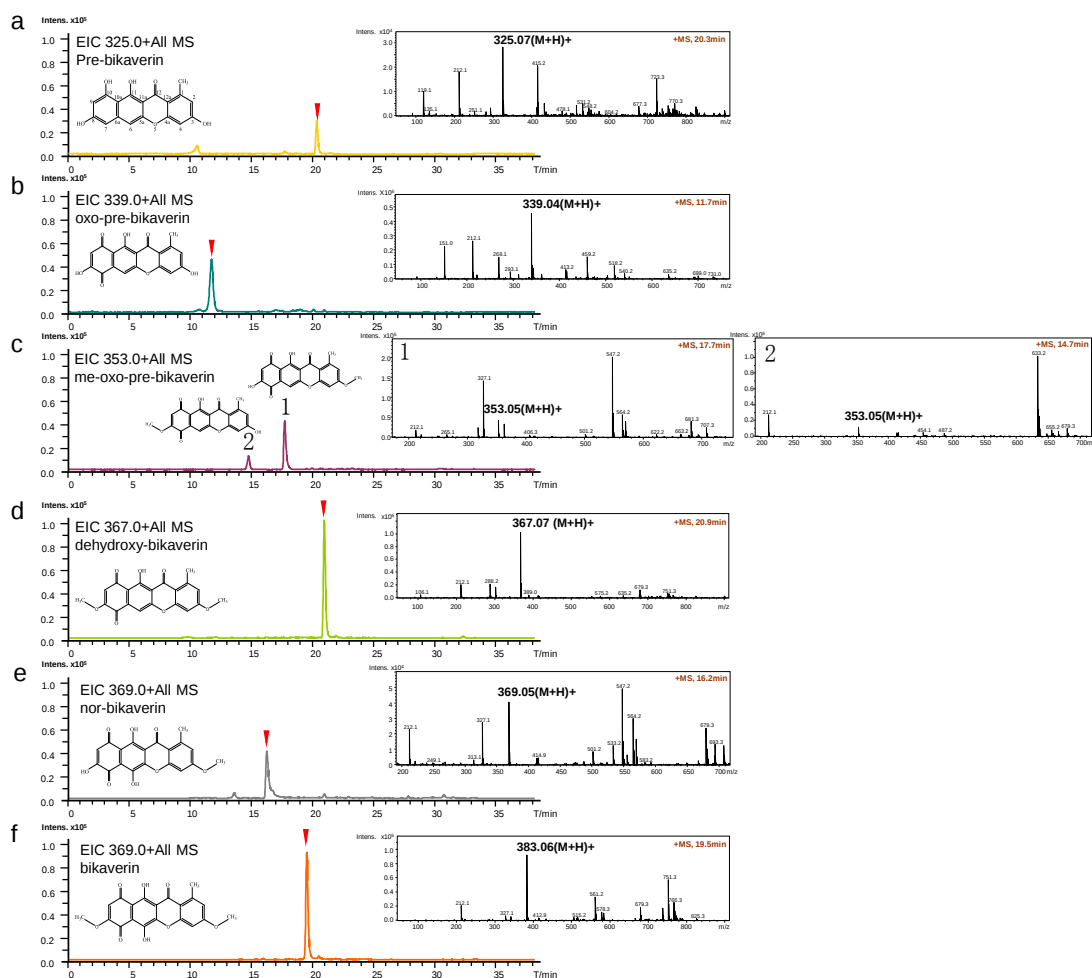


Supplementary Fig. 6. The EIC of oxo-pre-bikaverin and di-nor-bikaverin in strain yZM027. **a.** the peak of oxo-pre-bikaverin, and the corresponding mass spectrum. **b.** the extracted ion chromatogram of m/z 355.0, the mass of $[M-H]^+$ of di-nor-bikaverin.

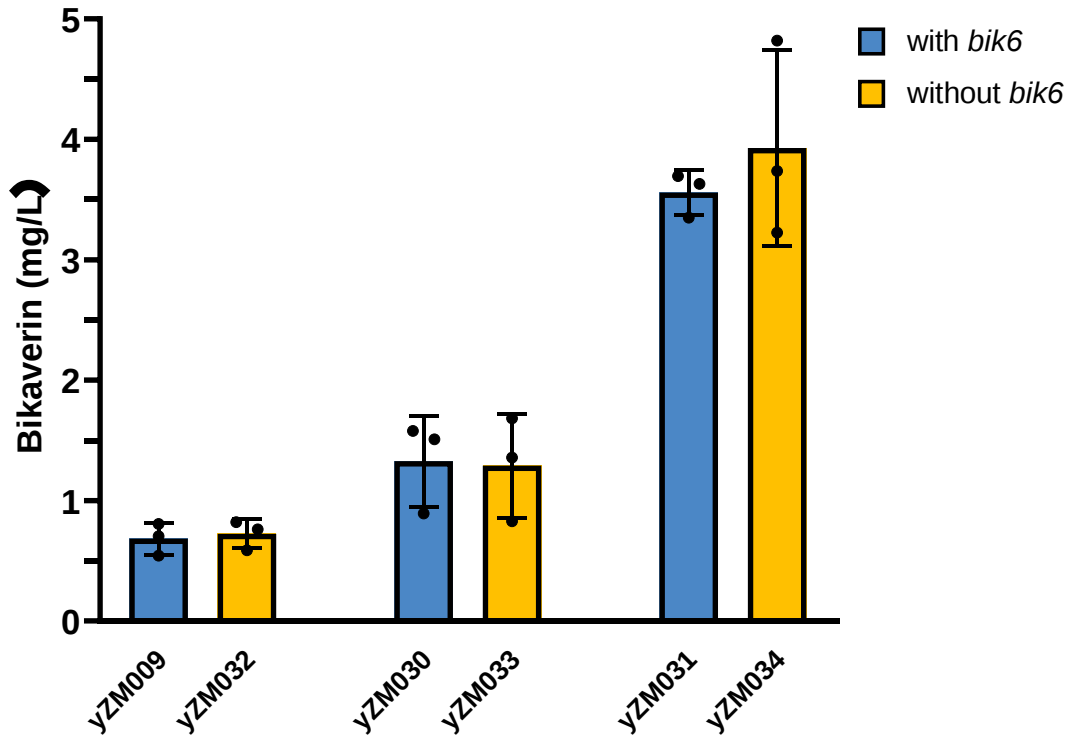
yZM028-EIC 339.0+All MS me-pre-bikaverin



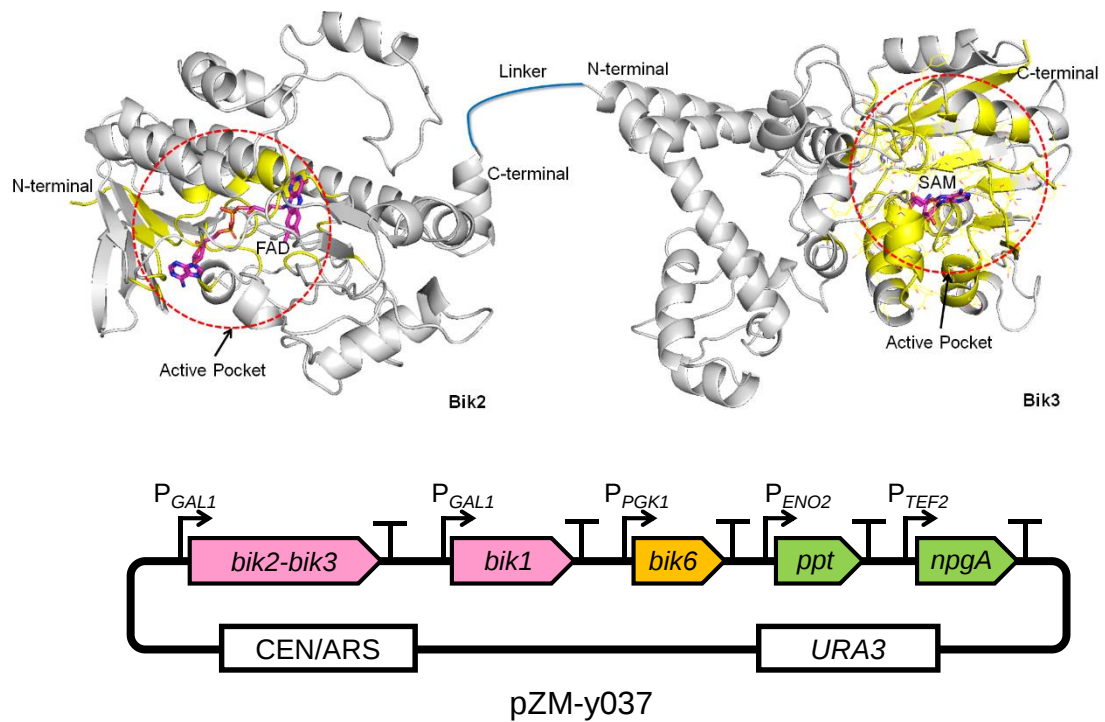
Supplementary Fig. 7. Me-pre-bikaverin detection from strain yZM028. **a.** The two peaks in EIC of me-pre-bikaverin (m/z 339 for $[M-H]^+$). **b.** The mass spectra of the two peaks.



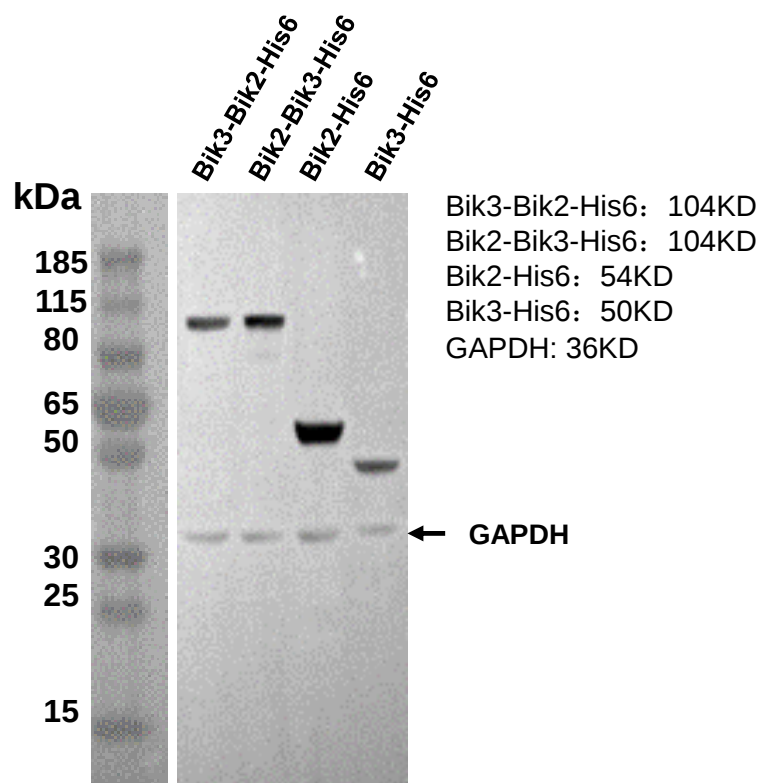
Supplementary Fig. 8. HPLC/ESI-MS spectrum of strain yZM009, with the complete bikaverin pathway. The extracted ion chromatograms and mass spectra of pre-bikaverin, oxo-pre-bikaverin, me-oxo-pre-bikaverin, dehydroxy-bikaverin, nor-bikaverin, bikaverin are shown in **a**, **b**, **c**, **d**, **e** and **f**, respectively.



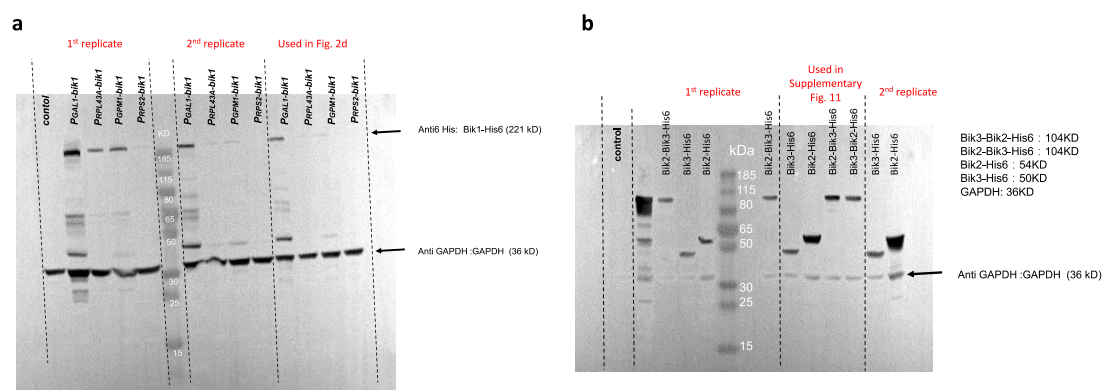
Supplementary Fig. 9. The effect of *bik6* deletion. Strains carrying bikaverin pathways were used to confirm that deletion of *bik6* had little effect on bikaverin production in *S. cerevisiae*. In each group, bikaverin pathways assembled are identical, except for *bik6*. Blue, no *bik6* in bikaverin pathways; yellow, *bik6* was included as the original design. yZM009 and yZM032: P_{GAL1} -*bik1*, P_{TEF1} -*bik2*, P_{TPII} -*bik3*; yZM030 and yZM033: P_{GAL1} -*bik1*, P_{TEF1} -*bik2*, P_{GAL1} -*bik3*; yZM031 and yZM034: P_{GAL1} -*bik1*, P_{GAL1} -*bik2*, P_{GAL1} -*bik3*. Data are presented as mean values $\pm$ SD and error bars show SD from n=3 biological replicates. Source data are provided as a Source Data file.



Supplementary Fig. 10. Homology modeling of Bik2 and Bik3. The homology modeling shows that the active pocket and FAD bind site of Bik2 are close to the N-terminal, while for Bik3, its SAM binding site and active pocket are near the C-terminal. In plasmid pZM-y037, proteins were fused in N-Bik2-Bik3-C direction, aiming to minimize the impact on the functions of Bik2 and Bik3.



Supplementary Fig. 11. Western blotting of Bik2, Bik3 and fusion proteins. Uncropped scans were supplied in the source data file. All the proteins were tagged with a 6×His-tag and driven by *GAL1* promoter. All strains were cultured in SC-Ura media with galactose as the carbon source. GAPDH were used as internal reference protein. Strain yZM044 was used for Bik3-Bik2-His6; strain yZM043 was used for Bik2-Bik3-His6; strain yZM045 was used for Bik2-His6; strain yZM046 was used for Bik3-His6. Experiment was repeated 3 times independently with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 12. The original uncropped scans of Western blot for Fig. 2 (a) and Supplementary Fig. 11 (b).

Supplementary Table 1. Yeast strains and coding plasmids for bikaverin pathway expressing in yeast.

Strain	Background	Plasmid	Description	Source
yZM006	BY4742	pZM-y006	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7}</i>	This study
yZM007	BY4742	pZM-y007	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPL43A}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7}</i>	This study
yZM008	BY4742	pZM-y008	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{GPM1}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7}</i>	This study
yZM009	BY4742	pZM-y009	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{GAL1}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7}</i>	This study
yZM010	BY4742	pZM-y010 (Bik1-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM006)</i>	This study
yZM011	BY4742	pZM-y011 (Bik2-GFP)	<i>pRS416_ P_{TEF1}-BIK2-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM006)</i>	This study
yZM012	BY4742	pZM-y012 (Bik3-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM006)</i>	This study
yZM013	BY4742	pZM-y013 (Bik6-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM006)</i>	This study
yZM014	BY4742	pZM-y014 (PPT-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM006)</i>	This study
yZM015	BY4742	pZM-y015 (NPGA-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPS2}-BIK1- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{HXT7} (derived from pZM006)</i>	This study
yZM016	BY4742	pZM-y016 (Bik1-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{RPL43A}-BIK1-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7} (derived from pZM007)</i>	This study
yZM017	BY4742	pZM-y017 (Bik1-GFP)	<i>pRS416_ P_{TEF1}-BIK2- T_{CYC1}-P_{TPH1}-BIK3- T_{ACS2}- P_{GPM1}-BIK1-GFP- T_{ADH1}- P_{TEF}-KanMX- T_{TEF}- T_{ACS1}-P_{PGK1}-BIK6- T_{ZEO1}- P_{ENO2}-PPT- T_{ADH2}-P_{TEF2}-NPGA- T_{HXT7}</i>	This study

			$T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM031)	
yZM041	BY4742	pZM-y041	$pRS416_P_{TEF1}\text{-BIK2- } T_{CYC1_P_{TPH1}\text{-BIK3- } T_{ACS2_P_{GAL1}\text{-BIK1- } T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1}}$	This study
yZM042	BY4742	pZM-y042	$pRS416_P_{GAL1}\text{-BIK3-BIK2- } T_{CYC1_P_{GAL1}\text{-BIK1- } T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$	This study
yZM043	BY4742		$pRS416_P_{GAL1}\text{-BIK2-BIK3-6xHis-tag- } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{CYC1_P_{GAL1}\text{-BIK1- } T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM037)	This study
yZM044	BY4742		$pRS416_P_{GAL1}\text{-BIK3-BIK2-6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{CYC1_P_{GAL1}\text{-BIK1- } T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM042)	This study
yZM045	BY4742		$pRS416_P_{GAL1}\text{-BIK2-6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{CYC1_P_{GAL1}\text{-BIK3- } T_{ACS2_P_{GAL1}\text{-BIK1- } GFP-T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM031)	This study
yZM046	BY4742		$pRS416_P_{GAL1}\text{-BIK2- } T_{CYC1_P_{GAL1}\text{-BIK3-6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{ACS2_P_{GAL1}\text{-BIK1- } GFP-T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM031)	This study
yZM047	BY4742		$pRS416_P_{TEF1}\text{-BIK2- } T_{CYC1_P_{TPH1}\text{-BIK3- } T_{ACS2_P_{RPS2}\text{-BIK1- } 6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM006)	This study
yZM048	BY4742		$pRS416_P_{TEF1}\text{-BIK2- } T_{CYC1_P_{TPH1}\text{-BIK3- } T_{ACS2_P_{GPM1}\text{-BIK1- } 6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM008)	This study
yZM049	BY4742		$pRS416_P_{TEF1}\text{-BIK2- } T_{CYC1_P_{TPH1}\text{-BIK3- } T_{ACS2_P_{RPL43A}\text{-BIK1- } 6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM007)	This study
yZM050	BY4742		$pRS416_P_{TEF1}\text{-BIK2- } T_{CYC1_P_{TPH1}\text{-BIK3- } T_{ACS2_P_{GAL1}\text{-BIK1-6xHis-tag - } T_{ADH1_P_{TEF}\text{-KanMX- } T_{TEF_T_{ACS1_P_{PGK1}\text{-BIK6- } T_{ZEO1_P_{ENO2}\text{-PPT- } T_{ADH2_P_{TEF2}\text{-NPGA- } T_{HXT7}}$ (derived from pZM009)	This study
yZM051	BY4742	pZM035+bZ M027		This study
yZM052	BY4742	pZM036+bZ M028		This study

Supplementary Table 2. *E. coli* strains and plasmids used in this study.

Plasmid	Description	Source
bZM007	<i>pUC19-VA1-P_{TEF1}-BIK2- T_{CYC1}-VA3</i>	This study
bZM008	<i>pUC19-VA3- P_{TPI1}-BIK3- T_{ACS2}-VA4</i>	This study
bZM009	<i>pUC19-VA4- P_{RPS2}-BIK1- T_{ACS1}-VA5</i>	This study
bZM010	<i>pUC19-VA5- P_{PGK1}-BIK6- T_{ZEO1}-VA6</i>	This study
bZM011	<i>pUC19-VA6- P_{ENO2}-PPT- T_{ADH2}-VA7</i>	This study
bZM012	<i>pUC19-VA7- P_{TEF2}-NPGA- T_{HXT7}-VA2</i>	This study
bZM013	<i>pUC19-VA6- P_{ENO2}-PPT- T_{ADH2}-VA2</i>	This study
bZM014	<i>pUC19-VA6- P_{ENO2}-NPGA- T_{ADH2}-VA2</i>	This study
bZM015	<i>pUC19-VA5- P_{ENO2}-PPT- T_{ADH2}-VA7</i>	This study
bZM016	<i>pUC19-VA5- P_{ENO2}-PPT- T_{ADH2}-VA7</i>	This study
bZM017	<i>pRS416-VA3-VA4- RFP-VA2</i>	This study
pJC170	<i>pRS416-VA1-RFP-VA2</i>	Mitchell <i>et al.</i> ¹
bZM019	<i>pUC19-VA1-P_{GAL1}-BIK2- T_{CYC1}-VA3</i>	This study
bZM020	<i>pUC19-VA3- P_{GAL1}-BIK3- T_{ACS2}-VA4</i>	This study
bZM021	<i>pUC19-VA4- P_{GAL1}-BIK1- T_{ACS1}-VA5</i>	This study
bZM023	<i>pUC19-VA4- P_{RPL43A}-BIK1- T_{ACS1}-VA5</i>	This study
bZM024	<i>pUC19-VA4- P_{GPM1}-BIK1- T_{ACS1}-VA5</i>	This study
bZM026	<i>pUC19-VA1-P_{GAL1}-BIK2-BIK3 -T_{CYC1}-VA3</i>	This study
bZM027	<i>pRS413- VA6- P_{ENO2}-PPT- T_{ADH2}-VA2</i>	This study
bZM028	<i>pRS413- VA6- P_{ENO2}-NPGA- T_{ADH2}-VA2</i>	This study
pGEV	<i>pGal4-ER-VP16-HIS3</i>	Cai <i>et al.</i> ² ; McIsaac <i>et al.</i> ³

Supplementary Table 3. Primer sequences used in this study.

Primer	Sequence
Bik2-GFP-F	<u>CCAGTTCAAGCTGCTACCGGTGTTGTTGAAGTTGGTTCT</u> GGTCGACGGATCCCCGGGT
Bik2-GFP-R	AACTAATTACATGATATCGACAAAGGAAAAGGGGCTGTTTCGATGAATTCGAGCTCGTT
Bik3-GFP-F	<u>TACGGTTCTTTTCATGTCTGTTATCGACGTTGTTTTGGGTGGT</u> CGACGGATCCCCGGGT
Bik3-GFP-R	CGAAATTTTATCTCATTACGAAATTTTCTCATTTAAGTTCGATGAATTCGAGCTCGTT
Bik1-GFP-F	<u>GCTTTGTGTGCTAAGATCAGAGAAACCATGGGTGTTAACGGT</u> CGACGGATCCCCGGGT
Bik1-GFP-R	AAAAAAAAAGTCGTCAATATAAAAAGGAAAGAAATCATCATCGATGAATTCGAGCTCGTT
Bik6-GFP-F	<u>ATCAGAGCTAGAGGTGAATTCTCTAAGTTGTCTACCTAC</u> GGTCGACGGATCCCCGGGT
Bik6-GFP-R	AAAGAACTTCTAGTAAAGTGCAGCACATTCAAGTGTGATCGATGAATTCGAGCTCGTT
PPT-GFP-F	<u>GAAGAAATCTTGGCTTTTCGGTGAACAAGCTTCTAAGCC</u> AGTCGACGGATCCCCGGGT
PPT-GFP-R	<u>AATGAAAACATAAAATCGTAAAGACATAAGAGATCCGCTT</u> CGATGAATTCGAGCTCGTT
NPGA-GFP-F	<u>ATCCAACCATGTGCTACCGGTGTTTGTAAGTTGTCTGGT</u> CGACGGATCCCCGGGT
NPGA-GFP-R	<u>ATTAGAGCGTGATCATGAATTAATAAAAAGTGTTCGCAA</u> ATCGATGAATTCGAGCTCGTT
His-tag-F	CACCACCATCACCATCATAAGCGAATTCTTATGATTTATGATTTTATTAT
His-tag-R	TCGATGAATTCGAGCTCGTT
Bik2-6xhis-F	<u>CCAGTTCAAGCTGCTACCGGTGTTGTTGAAGTTGGTTCT</u> CACCACCATCACCATCAC TAAGC
Bik2-6xhis-R	AACTAATTACATGATATCGACAAAGGAAAAGGGGCTGTTTCGATGAATTCGAGCTCGTT
Bik3-6xhis-F	<u>TACGGTTCTTTTCATGTCTGTTATCGACGTTGTTTTGGGT</u> CACCACCATCACCATCAC TAAGC
Bik3-6xhis-R	CGAAATTTTATCTCATTACGAAATTTTCTCATTTAAGTTCGATGAATTCGAGCTCGTT
Bik1-6xhis-F	<u>GCTTTGTGTGCTAAGATCAGAGAAACCATGGGTGTTAAC</u> CACCACCATCACCATCAC TAAGC
Bik1-6xhis-R	AAAAAAAAAGTCGTCAATATAAAAAGGAAAGAAATCATCATCGATGAATTCGAGCTCGTT
Bik6-6xhis-F	<u>ATCAGAGCTAGAGGTGAATTCTCTAAGTTGTCTACCTA</u> CACCACCATCACCATCAC TAAGC
Bik6-6xhis-R	AAAGAACTTCTAGTAAAGTGCAGCACATTCAAGTGTGATCGATGAATTCGAGCTCGTT
PPT-6xhis-F	<u>GAAGAAATCTTGGCTTTTCGGTGAACAAGCTTCTAAGCC</u> ACACCACCATCACCATCAC TAAGC
PPT-6xhis-R	<u>AATGAAAACATAAAATCGTAAAGACATAAGAGATCCGCTT</u> CGATGAATTCGAGCTCGTT
NPGA-6xhis-F	<u>ATCCAACCATGTGCTACCGGTGTTTGTAAGTTGTCT</u> CACCACCATCACCATCAC TAAGC
NPGA-6xhis-R	<u>ATTAGAGCGTGATCATGAATTAATAAAAAGTGTTCGCAA</u> ATCGATGAATTCGAGCTCGTT
Bik2-6xhis-F	<u>CCAGTTCAAGCTGCTACCGGTGTTGTTGAAGTTGGTTCT</u> CACCACCATCACCATCAC TAAGC
Bik2-6xhis-R	AACTAATTACATGATATCGACAAAGGAAAAGGGGCTGTTTCGATGAATTCGAGCTCGTT
Bik3-6xhis-F	<u>TACGGTTCTTTTCATGTCTGTTATCGACGTTGTTTTGGGT</u> CACCACCATCACCATCAC TAAGC
Bik3-6xhis-R	CGAAATTTTATCTCATTACGAAATTTTCTCATTTAAGTTCGATGAATTCGAGCTCGTT
Bik1-6xhis-F	<u>GCTTTGTGTGCTAAGATCAGAGAAACCATGGGTGTTAAC</u> CACCACCATCACCATCAC TAAGC
Bik1-6xhis-R	AAAAAAAAAGTCGTCAATATAAAAAGGAAAGAAATCATCATCGATGAATTCGAGCTCGTT

Note: The sequences underlined represent the homology arms used to promote yeast homologous recombination.

Supplementary References

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