Supplementary Tables and Figures for:

Heterozygous diploid and interspecies SCRaMbLEing

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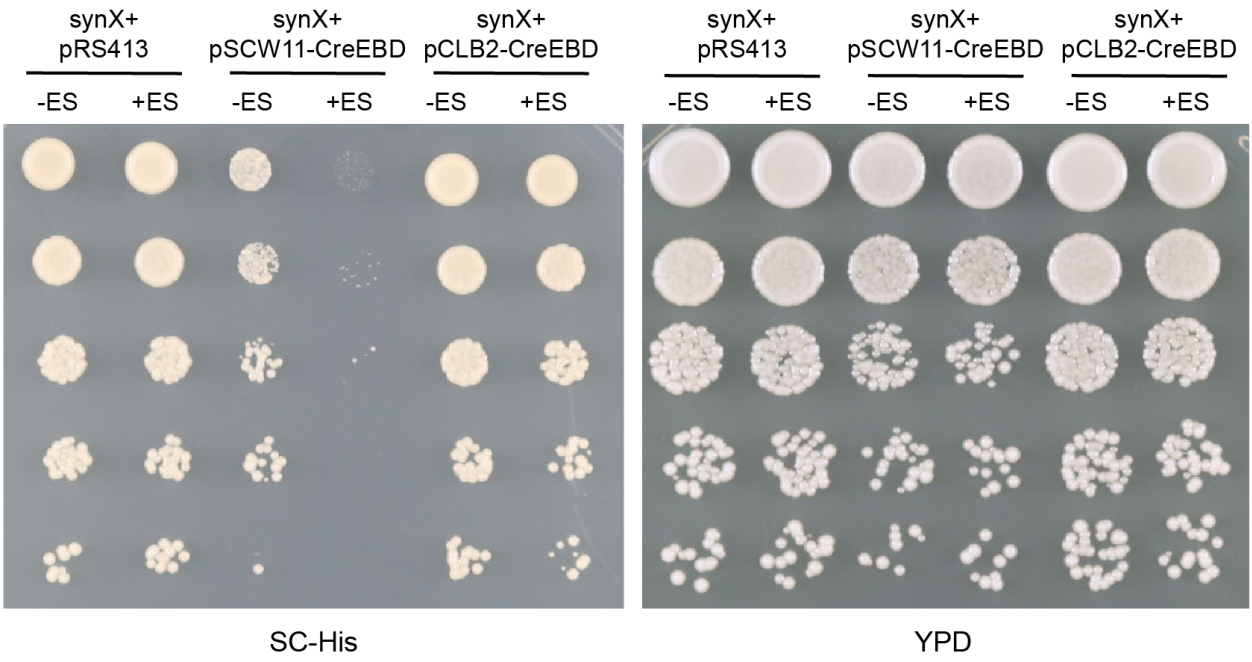
Supplementary Table 1: List of heterozygous diploid strains. Bold strains were used in this study.

Strain Name	Description	Genotype
yMS401	**synX and S288c**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS402	synX and UWOPS87-2421	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS403	synX and 378604X	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS404	synX and 273614N	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS405	synX and YIIc17_E5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS406	synX and Y55	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS407	synX and UWOPS83-787.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS408	synX and SK1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS409	synX and BC187	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS410	synX and YJM978	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS411	synX and YJM981	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS412	synX and YJM975	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS413	synX and DBVPG 1373	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS414	synX and DBVPG 1106	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS415	synX and DBVPG 6765	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS416	synX and L_1374	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS417	synX and L_1528	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS418	synX and DBVPG 6044	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS419	synX and NCYC 110	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS420	synX and UWOPS03-461.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS421	synX and UWOPS05-217.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS422	synX and UWOPS05-227.2	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS423	**synX and Y12**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*

yMS424	synX and YPS606	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS425	synX and YPS128	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS426	**synVsynX and S288c**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS427	synVsynX and UWOPS87-2421	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS428	synVsynX and 378604X	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS429	synVsynX and 273614N	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS430	synVsynX and YIIc17_E5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS431	synVsynX and Y55	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS432	synVsynX and UWOPS83-787.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS433	synVsynX and SK1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS434	synVsynX and BC187	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS435	synVsynX and YJM978	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS436	synVsynX and YJM981	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS437	synVsynX and YJM975	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS438	synVsynX and DBVPG 1373	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS439	synVsynX and DBVPG 1106	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS440	synVsynX and DBVPG 6765	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS441	synVsynX and L_1374	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS442	synVsynX and L_1528	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS443	synVsynX and DBVPG 6044	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS444	synVsynX and NCYC 110	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS445	synVsynX and UWOPS03-461.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS446	synVsynX and UWOPS05-217.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS447	synVsynX and UWOPS05-227.2	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS448	**synVsynX and Y12**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS449	synVsynX and YPS606	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS450	synVsynX and YPS128	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*

yMS501	synX and CBS432	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS502	synX and T21.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS503	synX and Y7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS504	synX and Q32.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS505	synX and Q59.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS506	synX and Q95.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS507	synX and S36.7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS508	synX and Z1.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS509	synX and Y6.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS510	synX and Q62.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS511	synX and Q89.8	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS512	synX and Y9.6	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS513	synX and Q74.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS514	synX and Q69.8	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS515	synX and W7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS516	synX and Q31.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS517	synX and Y8.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS518	synX and Z1.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS519	synX and Y8.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS520	synX and N-17	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS521	**synX and CBS 5829**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS522	synX and KPN3829	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS523	synX and YPS138	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS524	synX and DBVPG 6304	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS525	synX and A12	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS526	synX and N-44	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*
yMS527	synX and IFO 1804	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN10/WT10*

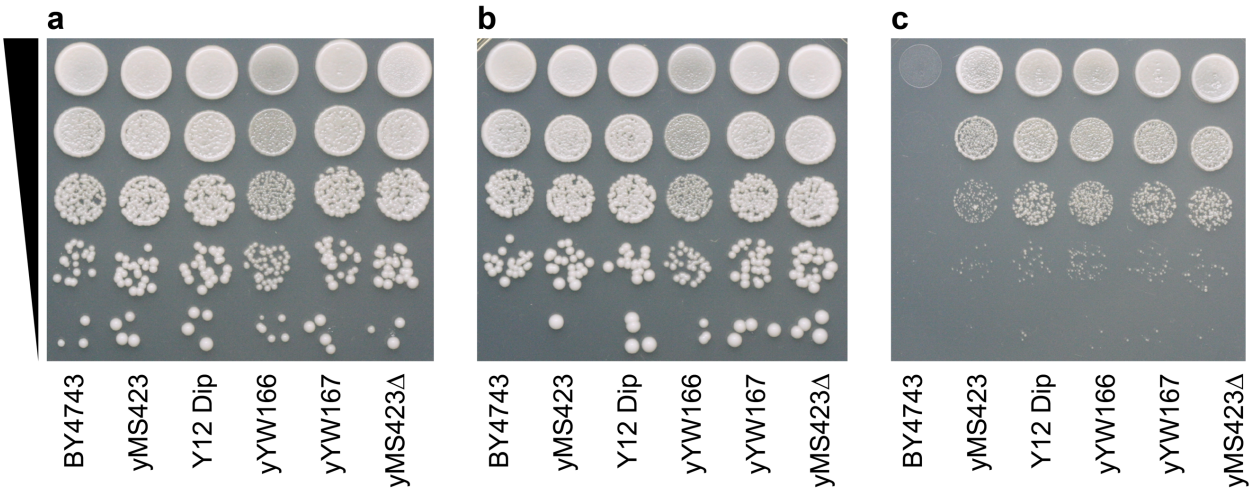
yMS528	synVsynX and CBS432	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS529	synVsynX and T21.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS530	synVsynX and Y7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS531	synVsynX and Q32.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS532	synVsynX and Q59.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS533	synVsynX and Q95.3	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS534	synVsynX and S36.7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS535	synVsynX and Z1.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS536	synVsynX and Y6.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS537	synVsynX and Q62.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS538	synVsynX and Q89.8	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS539	synVsynX and Y9.6	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS540	synVsynX and Q74.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS541	synVsynX and Q69.8	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS542	synVsynX and W7	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS543	synVsynX and Q31.4	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS544	synVsynX and Y8.5	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS545	synVsynX and Z1.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS546	synVsynX and Y8.1	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS547	synVsynX and N-17	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS548	**synVsynX and CBS 5829**	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS549	synVsynX and KPN3829	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS550	synVsynX and YPS138	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS551	synVsynX and DBVPG 6304	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS552	synVsynX and A12	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS553	synVsynX and N-44	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*
yMS554	synVsynX and IFO 1804	*MAT**a**/α ura3Δ0/ura3::KanMX ho::tR(ccu)J/ho::Hyg lys2::NatMX/LYS2 SYN5/WT5 SYN10/WT10*



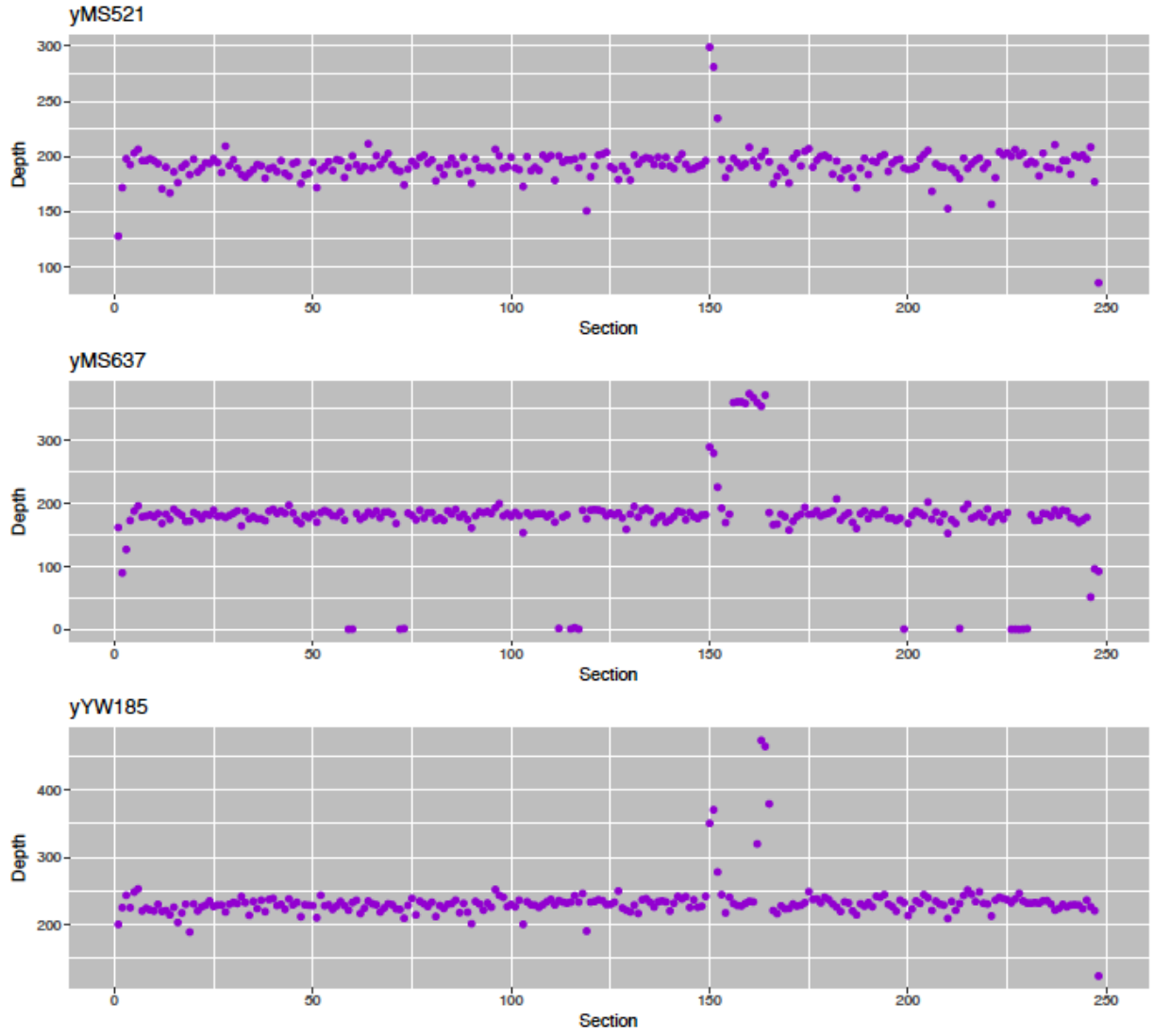
Supplementary Figure 1 Comparison of pSCW11 and pCLB2 promoters for Cre-EBD. Haploid strains bearing synX were transformed with the episomal shuttle vector pRS413 or pRS413 expressing Cre-EBD driven by either pSCW11 or pCLB2. Strains were subjected to SCRaMbLE for 8h in SC–His media without (–ES) or with 1μM β-estradiol (+ES) and were subsequently washed and plated as a serial dilution assay onto SC–His or YPD plates.

strain	location	structure variation	SCRaMbLEd segments	ORFs	size (bp)
yYW166	A	deletion	2-9	YJL222W-YJL217W	17031
	B	deletion	40-61	YJL161W-YJL130C	58298
	C	deletion	112-127	YJL052C-YJL028W	51548
	D	duplication	128-131	YJL027C-YJL022W	6544
	E	deletion	166	none	810
	F	deletion	194-248	YJR093C-YJR159W	137041
yYW167	G	deletion	47-53	YJL154C-YJL140W	20815
	H	deletion	73	none	386
	I	deletion	173-174	YJR055W-YJR056C	3548
	J	deletion	246-247	none	1047

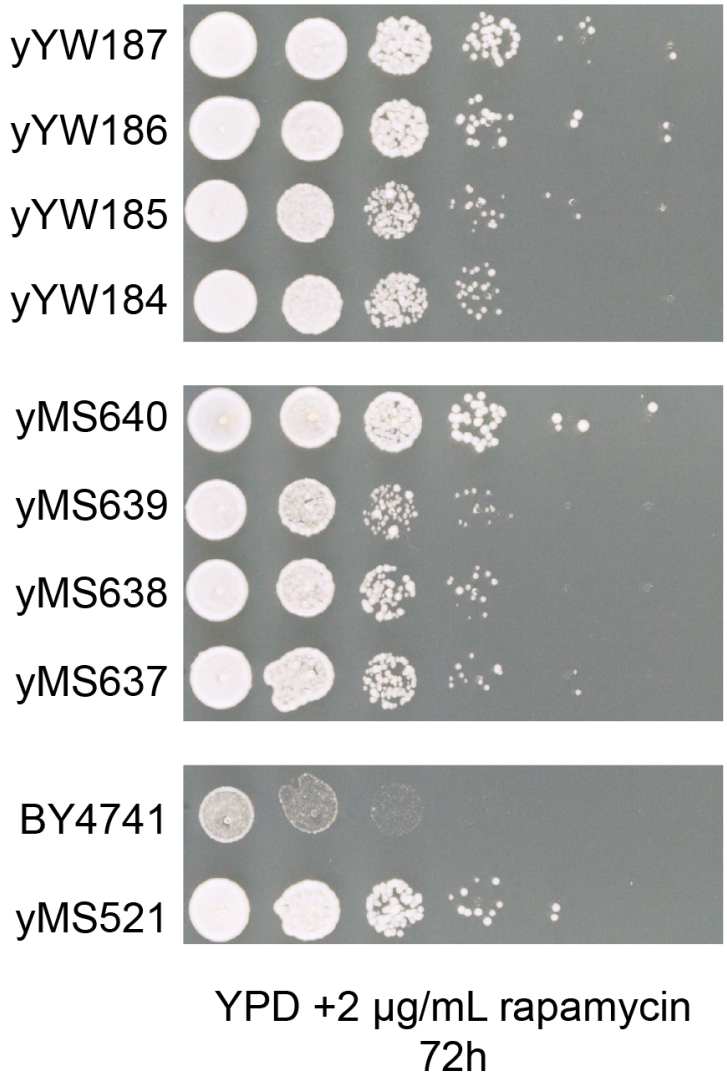
Supplementary Figure 2 Structural variation in yYW166 (blue) and yYW167 (red) reveals a common deletion of YJL154C-YJL140W among deletions ranging from 0.38 to 137 kb in length. The duplicated segment YJL027C-YJL022W is 6.5 kb in length.



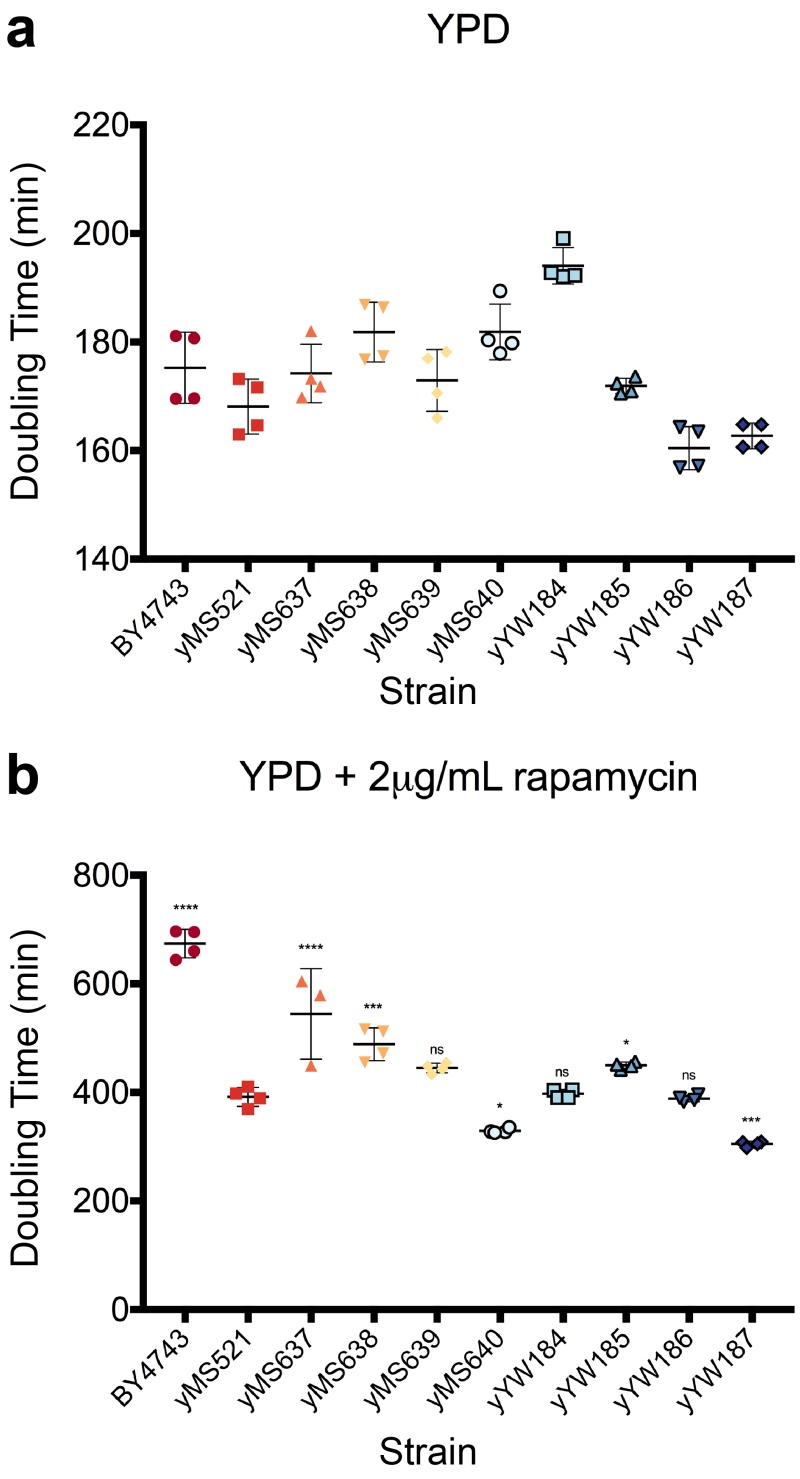
Supplementary Figure 3 Serial dilution assay showing growth on YPD of diploid strains BY4743, yMS423 (Y12-*synX* heterozygous diploid non-SCRaMbLEd parent), Y12 homozygous diploid, yYW166 (SCRaMbLEd yMS423), yYW167 (SCRaMbLEd yMS423), and yMS423Δ (yMS423 with deletion of YJL154C to YJL140W in *synX*) at (**a**) 30ºC, (**b**) 37ºC, and (**c**) 42ºC. Assays are shown after 48h of incubation.



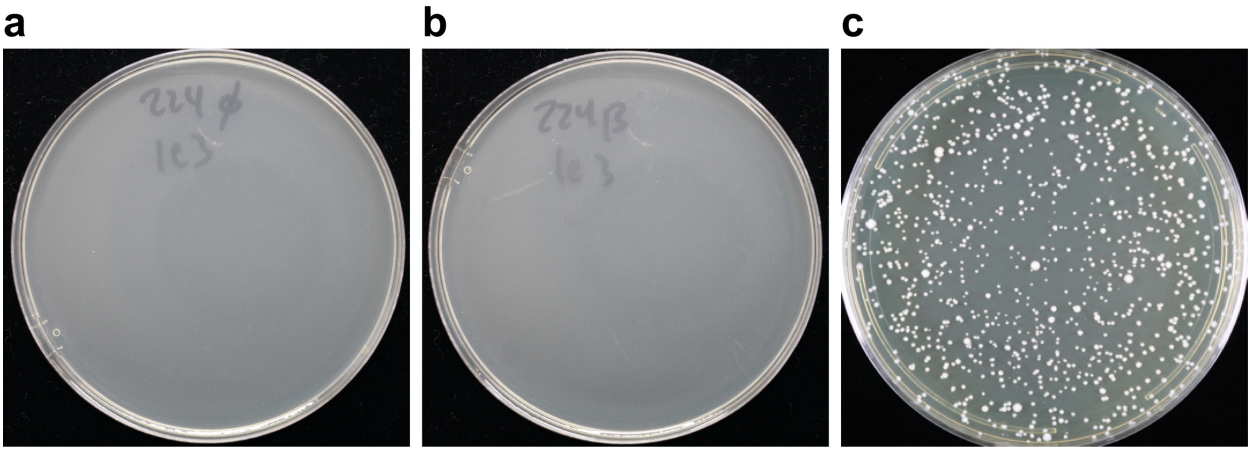
Supplementary Figure 4 Whole genome sequencing analysis revealed a common duplication region in both the SCRaMbLEd strains yMS637 and yYW185 compared to their non-SCRaMbLEd parent yMS521. This region, spanning two loxPsym-flanked segments and just over 2 kb in length, contains only the *POL32* gene.



Supplementary Figure 5 Growth of SCRaMbLEd, caffeine-tolerant CBS5829-*synX* and CBS5829-*synVsynX* strains in rapamycin.



Supplementary Figure 6 BY4743, yMS521, yMS637, yMS638, yMS639, yMS640, yYW184, yYW185, yYW186, and yYW187 were all grown in liquid YPD overnight and diluted to a starting A_{600} of 0.1 in either YPD or YPD with 2 μg/mL rapamycin. These strains were then cultured in a 96-well plate reader with shaking. Optical density measurements were taken every 10m and used to calculate doubling time. Error bars shown are mean and standard deviation from four technical replicates (three in the case of yMS637 in YPD with 2 μg/mL rapamycin). One-way ANOVA with multiple comparisons was used to assess difference between each sample and yMS521 (****: $p < 0.0001$, ***: $p < 0.001$, *: $p < 0.05$, ns: not significant).



Supplementary Figure 7 Growth after 96h of (**a**) non-SCRaMbLEd S288C-*synX* cells at 42ºC, (**b**) SCRaMbLEd S288C-*synX* cells at 42ºC, (**c**) SCRaMbLEd Y12-*synX* cells at 42ºC. Large colonies from the plate in (**c**) were picked for further analyses.

Supplementary Table 2: Variants shared between strains yMS637 and yYW185

Chromosome	Coordinate	Original	Variant	Notes
BY4741_chr01	27072	A	G	FLO9
BY4741_chr01	27075	G	A	FLO9
BY4741_chr01	27090	T	C	FLO9
BY4741_chr01	27099	T	C	FLO9
BY4741_chr01	27105	A	G	FLO9
BY4741_chr01	27126	T	C	FLO9
BY4741_chr01	27129	C	G	FLO9
BY4741_chr08	475929	TTA	T	low coverage
BY4741_chr13	65927	C	CTGT	NUP188
BY4741_chr14	522403	T	C	YNL054W-B transposable element
BY4741_chr14	522427	C	T	YNL054W-B transposable element
BY4741_chr14	522481	T	C	YNL054W-B transposable element
BY4741_chr14	522550	A	G	YNL054W-B transposable element
BY4741_chr14	522560	A	G	YNL054W-B transposable element
CBS5829.chr10	341311	TA	T	similar to Ty in S288C
CBS5829.chr10	707973	C	CAT	not in ORF
CBS5829.chr01	4837	CT	C	not in ORF
CBS5829.chr01	11602	A	AG	not in ORF
CBS5829.chr01	11948	G	A	YAL059C dubious ORF
CBS5829.chr01	16801	AGACATGGACT	A	GPB2
CBS5829.chr01	18213	A	T	not in ORF
CBS5829.chr01	21569	T	C	synonymous
CBS5829.chr01	24921	G	A	synonymous
CBS5829.chr01	26814	T	C	synonymous
CBS5829.chr01	30444	C	T	synonymous
CBS5829.chr01	34182	A	G	PTA1 K-->E
CBS5829.chr11	67624	T	C	MNN4 K-->R
CBS5829.chr11	67666	C	T	MNN4 R-->K
CBS5829.chr12	264204	ATT	A	low coverage
CBS5829.chr12	453171	G	GAT	low coverage
CBS5829.chr13	560747	A	T	not in ORF
CBS5829.chr13	780322	C	CA	not in ORF
CBS5829.chr13	798914	GA	G	not in ORF
CBS5829.chr14	634155	T	C	synonymous
syn10	232000	C	CA	not in ORF

The CBS5829 genome was downloaded from the Saccharomyces Genome Resequencing Project (http://www.sanger.ac.uk/research/projects/genomeinformatics/sgrp.html), while the *synX* sequence used was yeast_chr10_9_01 from BioStudio (http://54.160.105.26/gbrowse2/)

Supplementary Table 3: Inferred rearrangements in SCRaMbLEd strains

strain	segment 1	segment 2	depth	inferred rearrangement
yM637	10.129	10.-96	52	inversion
	10.131	10.97	52	
	10.-113	10.-111	52	deletion of segment 112
	10.-231	10.-225	51	deletion of segments 226-230
	10.-61	10.-58	47	deletion of segments 59 and 60
	10.-132	10.13	45	inversion
	10.-74	10.-71	45	deletion of segments 72 and 73
	10.212	10.214	43	deletion of segment 213
	10.-118	10.-114	43	deletion of segments 115-117
	10.164	10.156	35	duplication of segments 156-164
	10.-200	10.-198	22	deletion of segment 199
	10.02	10.-245	21	inversion
	10.-03	10.-01	13	deletion of segment 2
	10.245	10.247	13	deletion of segment 246
yMS638	10.-17	10.1	54	inversion
	10.16	10.-09	52	inversion
	10.-121	10.-119	44	deletion of segment 120
yMS639	10.-231	10.224	78	inversion
	10.-17	10.-15	52	deletion
	10.23	10.-223	40	inversion
yYW186	5.51	5.42	41	in parental strain
	5.116	5.-112	37	inversion
	5.-117	5.113	32	inversion
yYW187	10.-97	10.82	45	inversion
	5.51	5.42	36	in parental strain
	10.96	10.-81	26	inversion
yYW195	5.-48	5.-46	69	deletion
	5.12	5.07	56	deletion
	5.51	5.42	52	in parental strain
	5.-45	5.-43	46	deletion
yYW196	5.51	5.42	59	in parental strain