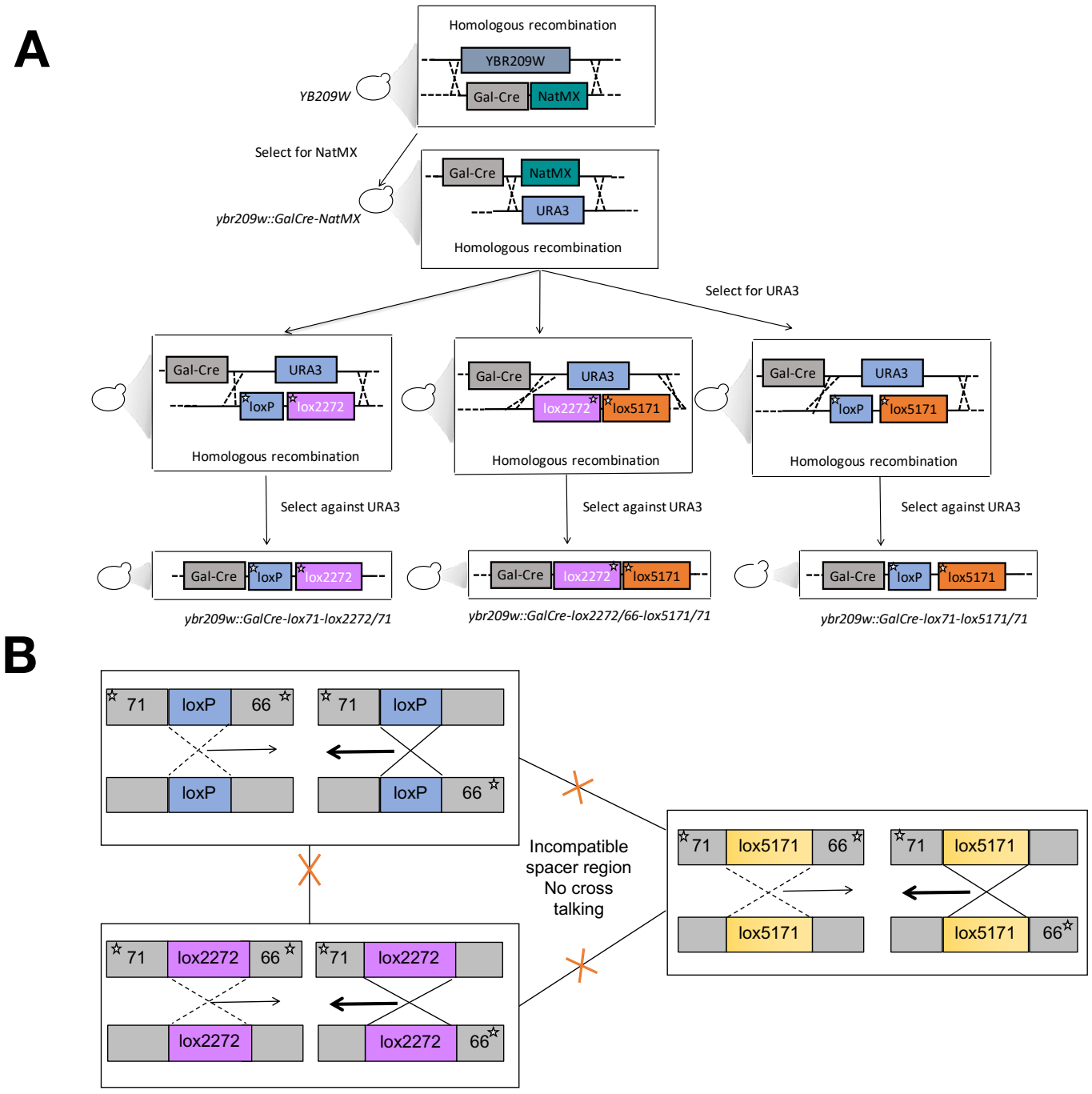

Figure S1. Related to Figure 1.

(A) Schematic of strain construction at the *YBR209W* locus. Lines with arrows indicate selection after transformation. The sequence at the *YBR209W* locus is indicated.

(B) The *loxP* variants used in this study. Three groups of *loxP* variants, which have different "core" regions, have a low rate of recombination with each other. The stars indicate mutations in the palindromic arms. The suffixes "71" and "66" in a *loxP* variant name (e.g. *lox71* or *lox2272/66*) indicate mutations in left and right arms, respectively.

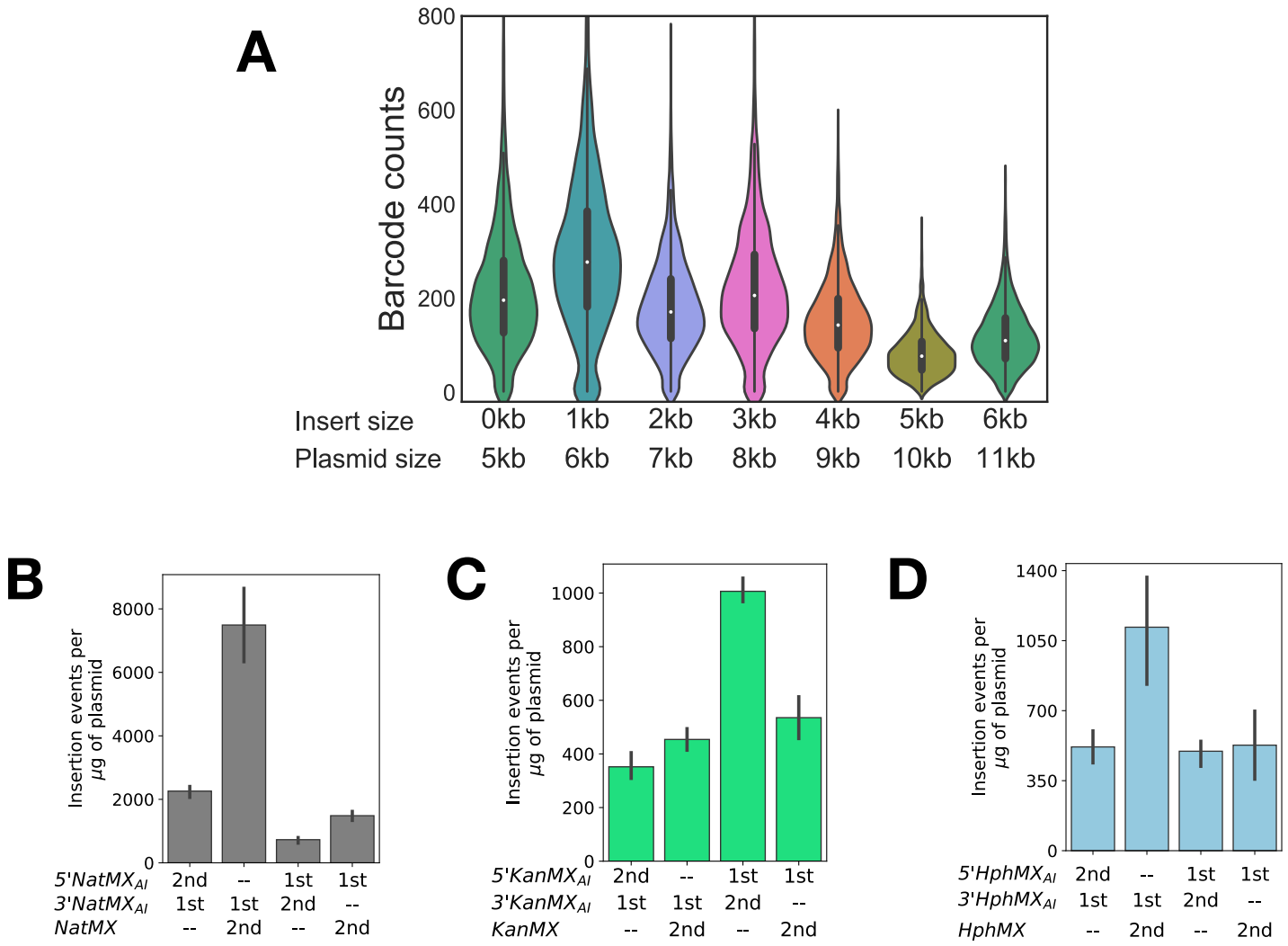

Figure S2. Related to Figure 1.

(A) Barcoded plasmids containing various size of inserts were pooled and integrated into yeast at the lox5171 site. Violin plots shows the distribution of barcode counts for each plasmid size. Each plasmid size contains at least 6,000 double barcodes. The width of each violin is fixed and violin areas are not proportional to the number of barcodes.

(B-D). Number of insertion events from transformations into yeast containing either *5'* or *3'* half of $NatMX_{AI}$ (B), $KanMX_{AI}$ (C), or $HphMX_{AI}$ (D) with plasmids containing either a full or half marker. 1st and 2nd indicates the order in which markers are inserted into the landing pad.

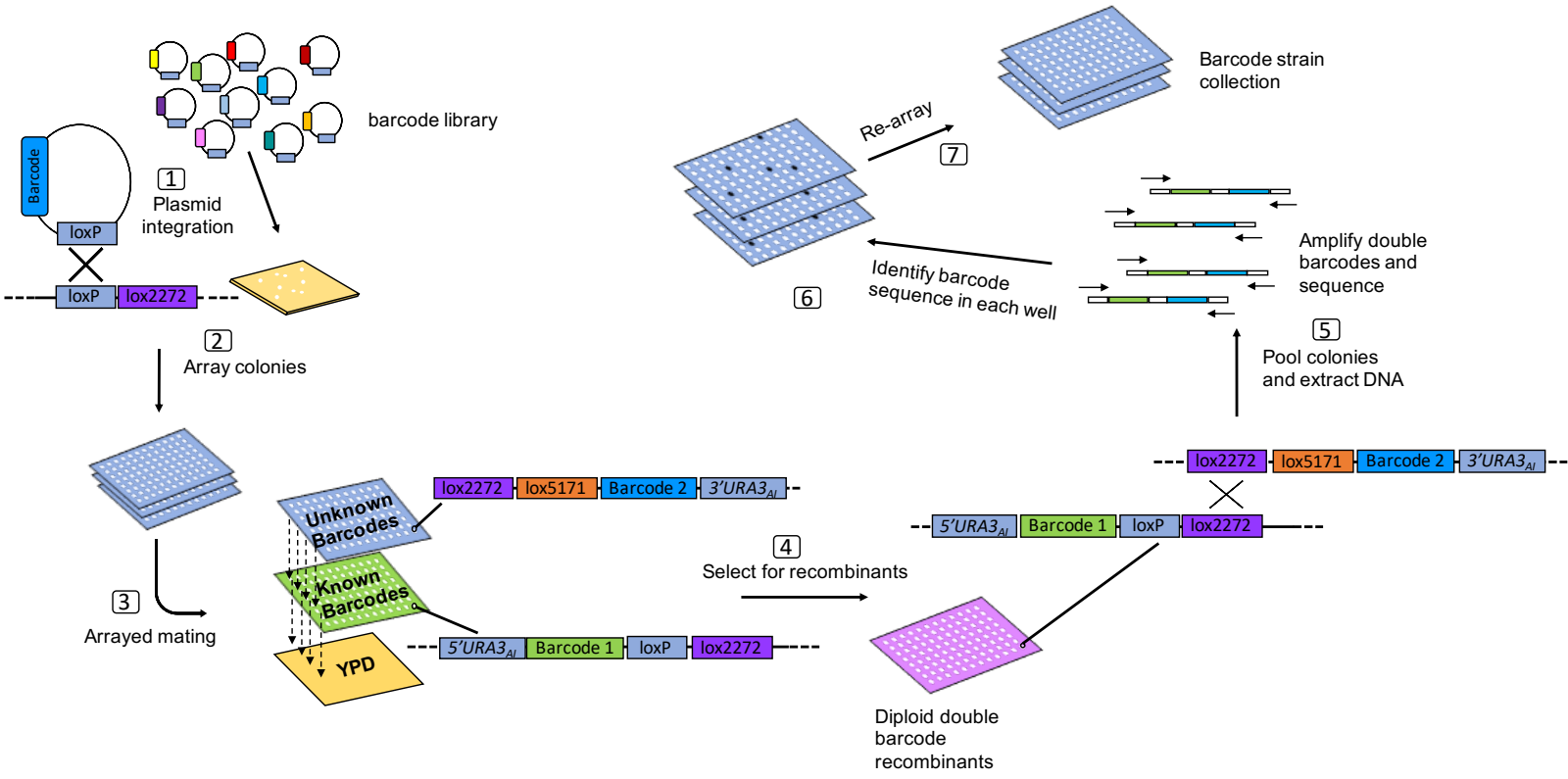

Figure S3. Related to Figure 2. REcombination-Directed Indexing (REDI) of complex yeast libraries. (1) Barcode plasmid libraries are integrated into the yeast genome at the landing pad locus via *Cre-loxP* recombination. (2) Successful transformants are picked and arrayed into multi-well plates. (3) Each transformant (containing an unknown barcode) mates with at least one strain containing a known barcode on YPD. (4) Recombination between homologous chromosome arms is induced and double barcode recombinants are selected. (5) Recombinants are pooled, genomic DNA is isolated, and double barcodes are PCR amplified and sequenced. (6) The known barcode sequence identifies the unknown barcode partner in each double barcode read. (7) After filtering out duplicate barcodes or wells that contain multiple unknown barcodes, each barcode is re-arrayed to form an arrayed strain collection.

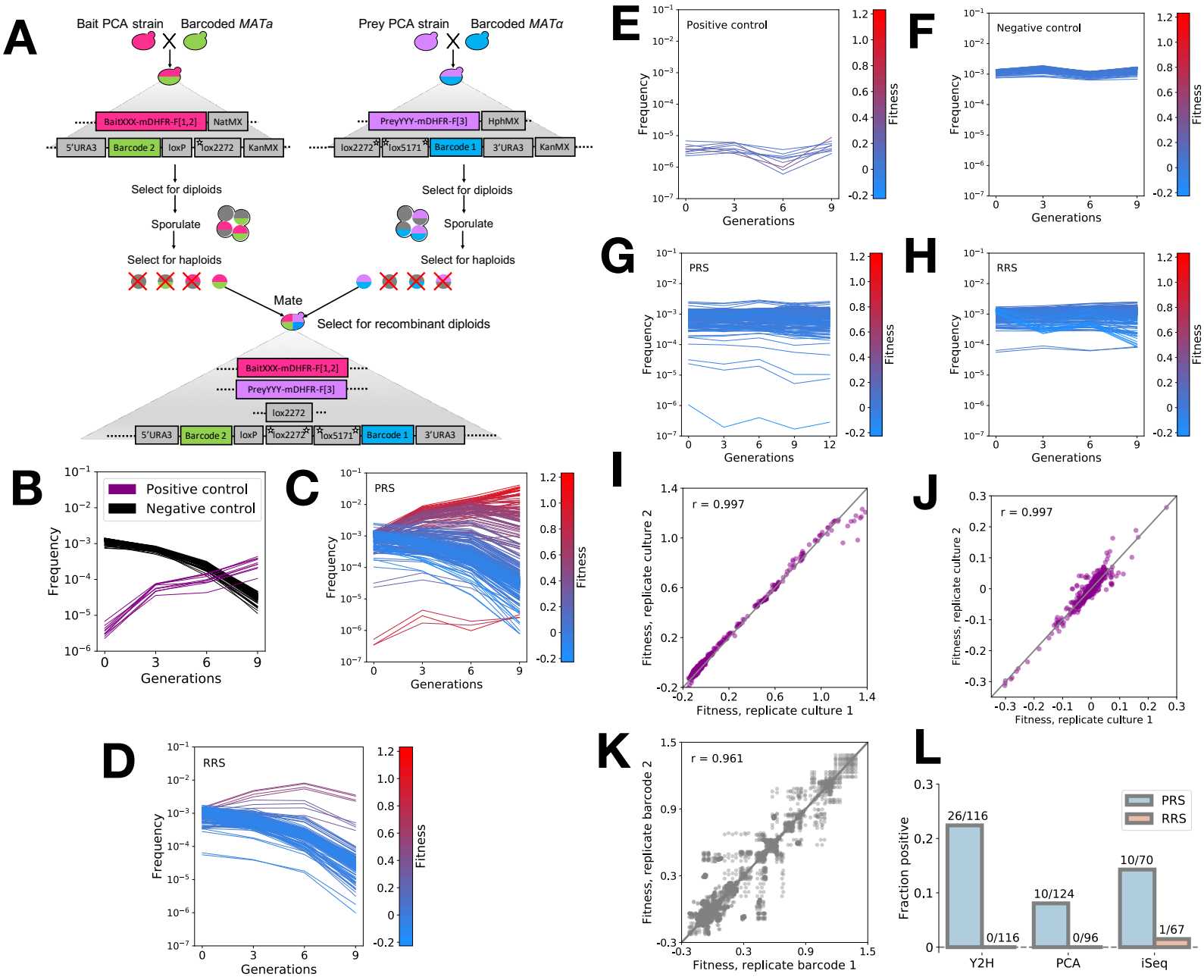

Figure S4. Related to Figure 2. Lineage trajectories of protein-protein interaction strains.

(A) Strains from the double barcoder collection (generated here) are pairwise mated to PCA strains from the protein interactome collection (Tarassov *et al*, 2008) and the resulting diploids are sporulated. Haploids of a specific mating type containing both the barcode and the PCA construct are selected for using a dual-SGA reporter (Tong *et al*, 2004), and selectable marker adjacent to the PCA construct and barcode. *MATa* and *MATα* haploids are mated in pairs. Recombination is induced in diploids to bring the two barcodes to the same chromosome and reconstitute the split *URA3* selectable marker. Barcoded positive (+mDHFR) and negative (-mDHFR) control strains are generated separately and spiked into the pool prior to competitive growth.

(B-D) Lineage trajectories of spiked-in positive (purple, 10 replicates) and negative (black, 100 replicates) control strains (B), and a set of 70 positive reference protein pairs (C, PRS) and 67 random reference protein pairs (D, RRS), each with 2-8 barcode replicates (Yu *et al*, 2008). Color for the PRS and RRS trajectories is the estimated fitness.

(E-H) Lineage trajectories of in the absence of methotrexate of 10 mDHFR+ positive control strains (E), 100 mDHFR- negative control strains (F), 69 positive reference protein pairs, PRS (G), and 67 random reference protein pairs, RRS (H). Each strain has 2-8 barcode replicates. Color of each trajectory indicates the estimated fitness.

(I) Scatter plot of the estimated fitnesses from replicate growth cultures in the presence of methotrexate (Pearson's r = 0.997).

(J) Scatter plot of the estimated fitnesses from replicate growth cultures in the absence of methotrexate (Pearson's r = 0.997).

(K) Scatter plot of the estimated fitnesses from replicate barcodes within a single culture that mark the same protein pair (Pearson's r = 0.961).

(L) Bar plot of the fraction of PRS and RRS protein pairs called as a PPI by yeast two-hybrid (Yu *et al*, 2008), traditional PCA (Tarassov *et al*, 2008), and iSeq. These values are rough estimates for the true positive and false positive rates of each assay, respectively.

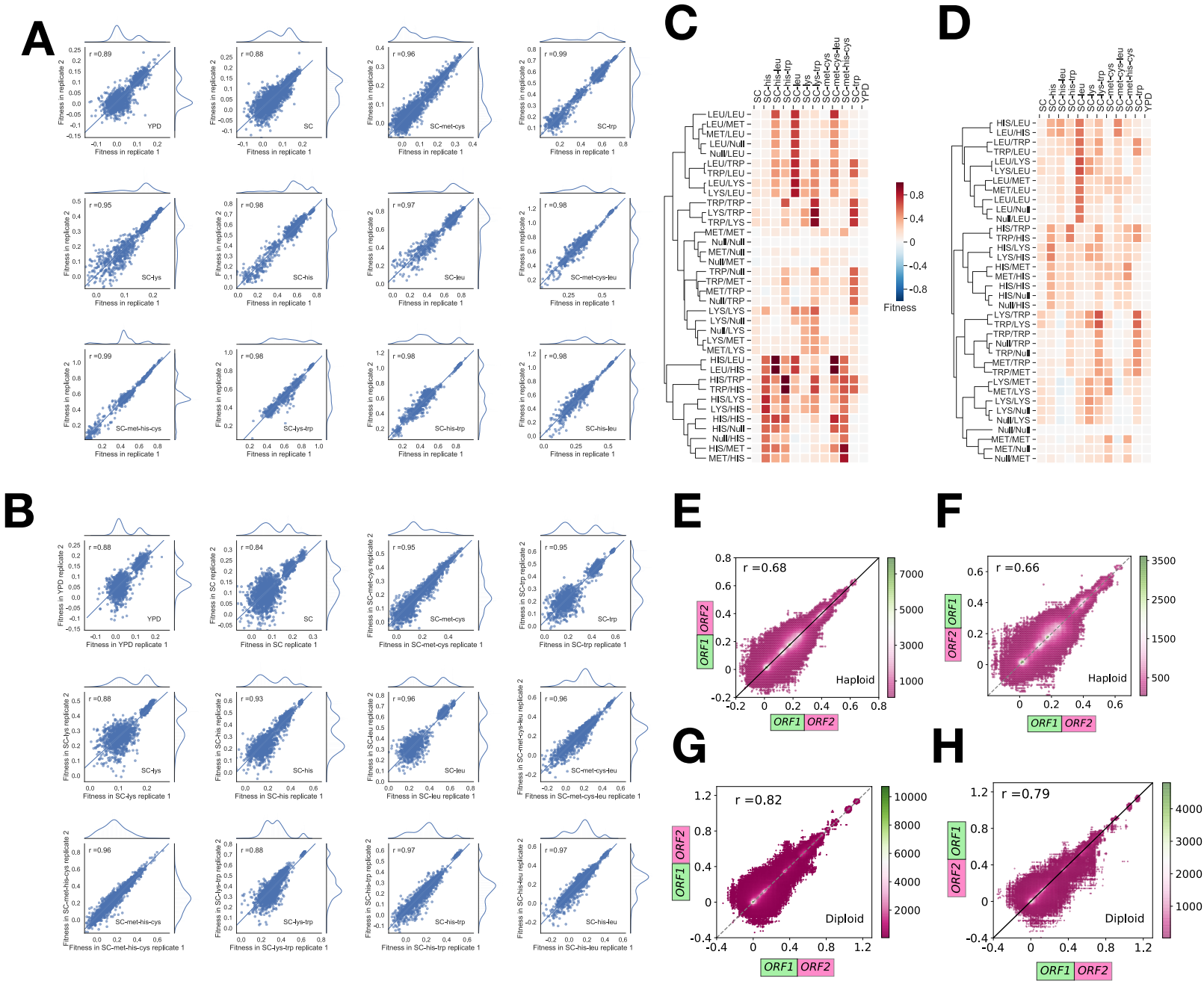

Figure S5. Related to Figure 2.

(A-B) Scatter plots of fitnesses estimated from replicate cultures in all 12 growth conditions for diploid (A) and haploid (B) libraries. r is the Pearson correlation coefficient.

(C-D) Heatmaps of the mean estimated fitness in diploid (C) and haploid (D) cells of each ORF pair in 12 growth conditions. Dendrograms are from hierarchical clustering.

(E-F) Hexmaps of fitnesses between yeast strains that carry different double barcodes but the same combination of ORFs for haploid libraries. Comparisons are within the same culture and between ORF pairs that are integrated at the iSeq landing pad in the same (E) or opposite (F) orientations

(G-H) Hexmaps of fitnesses between yeast strains that carry different double barcodes but the same combination of ORFs for diploid libraries. Comparisons are within the same culture and between ORF pairs that are integrated at the iSeq landing pad in the same (G) or opposite (H) orientations.

Table S1. Related to Figure 1. Sequences of loxP variants used in this study.

loxP variant	Spacer (5'- 3')	Left inverted repeat sequence	Right inverted repeat sequence
loxP	ATAACTTCGTATA	ATGTATGC	TATACGAAGTTAT
lox71	taccgTTCGTATA	ATGTATGC	TATACGAAGTTAT
lox66	ATAACTTCGTATA	ATGTATGC	TATACGAAcggta
lox5171	ATAACTTCGTATA	ATGTgTaC	TATACGAAGTTAT
lox5171/71	taccgTTCGTATA	ATGTgTaC	TATACGAAGTTAT
lox5171/66	ATAACTTCGTATA	ATGTgTaC	TATACGAAcggta
lox2272	ATAACTTCGTATA	AaGTATcC	TATACGAAGTTAT
lox2272/71	taccgTTCGTATA	AaGTATcC	TATACGAAGTTAT
lox2272/66	ATAACTTCGTATA	AaGTATcC	TATACGAAcggta

Table S2. Related to Figure 2. Barcoded plasmid libraries.

Plasmid library	loxP variant	Full marker	Half marker	Half marker exon length (bp)	Library size (x 10^6)
pBAR6-L1	lox5171/66	*KanMX*	*3'URA3$_{AI}$*	615	1.6
pBAR20-L1	lox66	*KanMX*	*5'URA3$_{AI}$*	189	2.8
pBAR7-L1	lox66	*HphMX*	*5'URA3$_{AI}$*	189	3.3
pBAR8-L1	lox5171/66	*NatMX*	*3'KanMX$_{AI}$*	216	0.38
pBAR9-L1	lox5171/66	*NatMX*	*3'HphMX$_{AI}$*	453	3.3
pBAR10-L1	lox66	*NatMX*	*5'KanMX$_{AI}$*	594	3.2
pBAR11-L1	lox66	*NatMX*	*5'HphMX$_{AI}$*	498	3.5
pBAR12-L1	lox66	*KanMX*	*5'HphMX$_{AI}$*	498	0.64
pBAR13-L1	lox66	*KanMX*	*5'NatMX$_{AI}$*	192	2.64
pBAR14-L1	lox5171/66	*KanMX*	*3'HphMX$_{AI}$*	453	0.35
pBAR15-L1	lox5171/66	*KanMX*	*3'NatMX$_{AI}$*	381	0.76
pBAR16-L1	lox5171/66	*HphMX*	*3'NatMX$_{AI}$*	381	0.19
pBAR17-L1	lox5171/66	*HphMX*	*3'KanMX$_{AI}$*	216	0.29
pBAR18-L1	lox66	*HphMX*	*5'NatMX$_{AI}$*	192	1
pBAR19-L1	lox66	*HphMX*	*5'KanMX$_{AI}$*	594	0.12

Table S3. Related to Figure 2. Oligonucleotides used in this study.

Primers for amplifying barcodes with loxP variants	
pXL005	CCAGCTGGTACCNNNNNAANNNNNTTNNNNNTTNNNNNATAACTTCGTATAATGTATGCT
pXL006	CCAGCTGGTACCNNNNNAANNNNNAANNNNNTTNNNNNTTACCGTTCGTATAGTACACA
pXL296	CCAGCTAGATCTNNNNNAANNNNNTTNNNNNTTNNNNNATAACTTCGTATAATGTATGCT
pXL297	CCAGCTGGATCCNNNNNAANNNNNAANNNNNTTNNNNNTACCGTTCGTATAGTACACAT
p23	GCCGAAATTGCCAGGATCAGG
Primers for Gibson assembly ORFs into barcode plasmids	
pXL094	GCCGCTTAATTAACAATTGGAGATTGTACTGAGAGTGCAC
pXL095	gTCTAGACCTAGGCGTACGTCTGTGCGGTATTTCACACCG
pXL096	GCCGCTTAATTAACAATTGGGGCTTCTCTTATGGCAACCG
pXL097	gTCTAGACCTAGGCGTACGTGGAACCCTAGTGTTGAATGGC
Primers for amplify fragments from human intron KCNIP4	
pXL267	CCAGCTTCTAGAatccttggacttgccatttg
pXL268	CCAGCTTCTAGAagcttggcaccagactcact
pXL269	CCAGCTACTAGTggatttgaatgccttcctga
pXL270	CCAGCTACTAGTtcatcccgaccaccaataat
pXL271	CCAGCTCAATTGGCCACACAGGAAAAAGGAAA
pXL272	CCAGCTCAATTGCTGGCAGAAAGTAGCCAAGG
Yeast cloning	
pEV8	GTTCTTTGCTTTTTTTCCCCAACGACGTCGAACACATTAGTCCTACGCACTTAACTTCGCA
pEV9	GCTTGCGCTAACTGCGAACAGAGTGCCCTATGAAATAGGGGAATGCATATCATACGTAAT
p14	GCGAACAGAGTAAACCGAA
p15	GAAGGTCTGAAGGAGTTC
pXL003	ATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGGAGATTGTACT
pXL004	AACATGTTCTTTGCTTTTTTTCCCCAACGACGTCGAACACATTAGTCCTACTGTGCGGTAT
pXL008	AGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGG*TACCGTTC* *GTATAATGTATGCTATACGAAGTTAT*TGCGCGGTGATCACTTATGG*TACCGTTCGTATAATGTGT*
pXL043	AGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGG*TACCGTTC* *GTATAATGTATGCTATACGAAGTTAT*TGCGCGGTGATCACTTATGG*TACCGTTCGTATAAAGTAT*
pXL044	AGATCTGTTTAGCTTGCCTCGTCCCCGCCGGGTCACCCGGCCAGCGACATGG*ATAACTTC* *GTATAAAGTATCCTATACGAACGGTA*TGCGCGGTGATCACTTATGG*TACCGTTCGTATAATGTG*
Primers for barcoded PPI construction	
pZL065	CGGGATCCATGGGCGGTGGCGGATCAGGAGG
pZL070	CCGCTCGAGTCGACACTGGATGGCGGCGT
pZL071	TCAGGAGGCGGTGGGTCT
pZL072	CCGACTATCCAAACCATGTCTACTTTACTGGTACCCAATTCCGGTTGTTCAAT
pZL073	AGTAAAGTAGACATGGTTTGGATAGTCGG
pZL074	AGACCCACCGCCTCCTGA

Table S3 (Continued). Related to Figure 2. Oligonucleotides used in this study.

gblocks for split drug resistant markers construction	
5'HphMX	CCAGCTCCATGTCGCTGGCCGGGTGACCGATTCGGTAATCTCCGAACAGAGTCTTGACGTGCGCAGCTCAGGGGCATGAT GTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACATTTTGATGGCCGCACGGCGCGA AGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACAGACGCGTTGAATTGTCCCCACGC CGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATATACTTCCTTTTAAAATCTTGCTAG GATACAGTTCTCACATCACATCCGAACATAAACAACCATGGGTAAAAAGCCTGAACTCACCGCGACGTCTGTCGAGAAGT TTCTGATCGAAAAGTTCGACAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGCTTCGATG TAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATCGGCACTTTGC ATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGGAATTCAGCGAGAGCCTGACCTATTGCATCTCCCGCCGTGC ACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCCATGGATGCGA TCGCTGCGGCCGATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGT GATTTCATATGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCGC AGgtaTGTTAATATGGACTAAAGGAGGCTTTTGTCGACGGATCCGATATCGGTACCCTCCTGCTTAAGGGCGCGCCAGCTGG
3'HphMX	CCAGCTGGCGCGCCCTTAAGCAGGAGGGTACCGATATCAGATCTAAGCTTGAATTCGAATTTTTACTAACAAATGGTATTAT TTATAAcagGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCT CCAACAATGTCCTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCCCAATAC GAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCC GGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGG CAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGGACTGTCGGGCGTACAC AAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTACTCGCCGATAGTGGAAACCGACGCCCCAG CACTCGTCCGAGGGCAAAGGAATAATCAGTACTGACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTT TTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAA GTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGC CGCCATCCAGTGTCGAAAACGAGCTCCATTAGTGAGTAACTCTGTGATATCTCTCTATAATTAGCAGTTTTTCACTGAAATT CCAGGAAAGGTAATAAACTCAGATTTTTTTTTATACTATTGGCTGCTTGTTACTTATATATCTTGAACTTCTCCCAGCGGGTC TTCAAATACATTTGGGCGATGTTCATGTTCATTAGGCAGGTATTTCGACATTGAGTCACACGCGAAAAACCGCCGGAATTTT TTATGTAATTGCAAGTGGAATTCCGCTGGCAAAACTATTGGGCCCGTCACCCGGaccGGTCACCCGGCCAGCGACATGGAG CTGG
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3'KanMX	CCAGCTGGCGCGCCCTTAAGCAGGAGGGTACCGATATCAGATCTAAGCTTGAATTCGAATTTTTACTAACAAATGGTATTAT TTATAAcagcttgataaccttatttttgacgaggggaaattaataggttgtattgatgttggacgagtcggaatcgcagaccgataccaggatcttgccatcctatggaactgcctcggtgagttttctc cttcattacagaaacggctttttcaaaaatatggtattgataatcctgatatgaataaattgcagtttcatttgatgctcgatgagtttttctaaTCAGTACTGACAATAAAAAGATTCTT GTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTTAGCGTGATTTATATTTTTTT TCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCAATCGTATGTGAATGCTGGTC GCTATACTGCTGTCGATTCGATACTAACGCCGCCATCCAGTGTCGAAAACGAGCTCCATTAGTGAGTAACTCTGTGATATCT CTCTATAATTAGCAGTTTTTCACTGAAATTCCAGGAAAGGTAATAAACTCAGATTTTTTTTTATACTATTGGCTGCTTGTTACT TATATATCTTGAACTTCTCCCAGCGGGTCTTCAAATACATTTGGGCGATGTTCATGTTCATTAGGCAGGTATTTCGACATTGA GTCACACGCGAAAAACCGCCGGAATTTTTTATGTAATTGCAAGTGGAATTCCGCTGGCAAAACTATTGGGCCCGTCACCCG GaccGGTCACCCGGCCAGCGACATGGAGCTGG
5'NatMX	CCAGCTCCATGTCGCTGGCCGGGTGACCGATTCGGTAATCTCCGAACAGAGGCCCAGAATACCCTCCTTGACAGTCTTGAC GTGCGCAGCTCAGGGGCATGATGTGACTGTCGCCCGTACATTTAGCCCATACATCCCCATGTATAATCATTTGCATCCATACA TTTTGATGGCCGCACGGCGCGAAGCAAAAATTACGGCTCCTCGCTGCAGACCTGCGAGCAGGGAAACGCTCCCCTCACA GACGCGTTGAATTGTCCCCACGCCGCGCCCCTGTAGAGAAATATAAAAGGTTAGGATTTGCCACTGAGGTTCTTCTTTCATA TACTTCCTTTTAAAATCTTGCTAGGATACAGTTCTCACATCACATCCGAACATAAACAACCATGGGTACCACTCTTGACGAC ACGGCTTACCGGTACCGCACCAGTGTCCCGGGGGACGCCGAGGCCATCGAGGCACTGGATGGGTCCTTCACCACCGACAC CGTCTTCCGCGTCACCGCCACCGGGGACGGCTTCACCCTGCGGGAGGTGCCGGTGGACCCGCCCCTGACCAAGGTGTTCC CCGACGACGAAgtaTGTTAATATGGACTAAAGGAGGCTTTTGTCGACGGATCCGATATCGGTACCCTCCTGCTTAAGGGCGC GCCAGCTGG
3'NatMX	CCAGCTGGCGCGCCCTTAAGCAGGAGGGTACCGATATCAGATCTAAGCTTGAATTCGAATTTTTACTAACAAATGGTATTAT TTATAAcagTCGGACGACGAATCGGACGACGGGGAGGACGGCGACCCGGACTCCCGGACGTTCGTCGCGTACGGGGACGA CGGCGACCTGGCGGGCTTCGTGGTCGTCTCGTACTCCGGCTGGAACCGCCGGCTGACCGTCGAGGACATCGAGGTCGCCC CGGAGCACCGGGGGCACGGGGTCGGGCGCGCGTTGATGGGGCTCGCGACGGAGTTCGCCCGCGAGCGGGGCGCCGGGC ACCTCTGGCTGGAGGTCACCAACGTCAACGCACCGGCGATCCACGCGTACCGGCGGATGGGGTTCACCCTCTGCGGCCTG GACACCGCCCTGTACGACGGCACCGCCTCGGACGGCGAGCAGGCGCTCTACATGAGCATGCCCTGCCCCTAATCAGTACT GACAATAAAAAGATTCTTGTTTTCAAGAACTTGTCATTTGTATAGTTTTTTTATATTGTAGTTGTTCTATTTTAATCAAATGTT AGCGTGATTTATATTTTTTTTCGCCTCGACATCATCTGCCCAGATGCGAAGTTAAGTGCGCAGAAAGTAATATCATGCGTCA ATCGTATGTGAATGCTGGTCGCTATACTGCTGTCGATTCGATACTAACGCCGCCATCCAGTGTCGAAAACGAGCTCCATTAG TGAGTAACTCTGTGATATCTCTCTATAATTAGCAGTTTTTCACTGAAATTCCAGGAAAGGTAATAAACTCAGATTTTTTTTTA TACTATTGGCTGCTTGTTACTTATATATCTTGAACTTCTCCCAGCGGGTCTTCAAATACATTTGGGCGATGTTCATGTTCATTA GGCAGGTATTTCGACATTGAGTCACACGCGAAAAACCGCCGGAATTTTTTATGTAATTGCAAGTGGAATTCCGCTGGCAAA ACTATTGGGCCCGTCACCCGGaccGGTCCAGCGACATGGAGCTGG

Table S3 (Continued). Related to Figure 2. Oligonucleotides used in this study.

Forward primers for EVO1 cycle	
P104	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCGATGTTTAATATGGACTAAAGGAGGCTTTT
P105	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNACAGTGTTAATATGGACTAAAGGAGGCTTTT
P111	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTGACCATTAATATGGACTAAAGGAGGCTTTT
P112	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGCCAATTTAATATGGACTAAAGGAGGCTTTT
P122	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNATCACGTTAATATGGACTAAAGGAGGCTTTT
P124	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGGCTACTTAATATGGACTAAAGGAGGCTTTT
P125	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTAGCTTTTAATATGGACTAAAGGAGGCTTTT
P130	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTCTCCCTTAATATGGACTAAAGGAGGCTTTT
P131	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGACGTCTTAATATGGACTAAAGGAGGCTTTT
P132	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGAGGTTTTAATATGGACTAAAGGAGGCTTTT
P135	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGTTAACTTAATATGGACTAAAGGAGGCTTTT
P137	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNAACCACTTAATATGGACTAAAGGAGGCTTTT
P201	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGCTTGATTAATATGGACTAAAGGAGGCTTTT
P202	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCAAGAATTAATATGGACTAAAGGAGGCTTTT
P203	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNATGCGATTAATATGGACTAAAGGAGGCTTTT
P204	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCATAGGTTAATATGGACTAAAGGAGGCTTTT
P205	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTTTTTGTTAATATGGACTAAAGGAGGCTTTT
P206	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTGGAAGTTAATATGGACTAAAGGAGGCTTTT
P207	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCCGGGCTTAATATGGACTAAAGGAGGCTTTT
P208	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCTACTCTTAATATGGACTAAAGGAGGCTTTT
P209	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNAGTGATTTAATATGGACTAAAGGAGGCTTTT
P210	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNTTCGGTTTAATATGGACTAAAGGAGGCTTTT
P211	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNGAATCGTTAATATGGACTAAAGGAGGCTTTT
P212	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNNCGCATATTAATATGGACTAAAGGAGGCTTTT
Reverse primers for EVO1 cycle	
P101	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTATATACGCTCGAATTCAAGCTTAGATCTGATA
P108	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCGCTCTATCTCGAATTCAAGCTTAGATCTGATA
P109	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGAGACGTCTTCGAATTCAAGCTTAGATCTGATA
P110	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNATACTGCGTTCGAATTCAAGCTTAGATCTGATA
P126	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNACTAGCAGATCGAATTCAAGCTTAGATCTGATA
P127	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTGAGCTAGCTCGAATTCAAGCTTAGATCTGATA
P128	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCTGCTACTCTCGAATTCAAGCTTAGATCTGATA
P129	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCGTACGCATCGAATTCAAGCTTAGATCTGATA
P133	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTCTCCGCTCGAATTCAAGCTTAGATCTGATA
P134	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTTAATCCTCTCGAATTCAAGCTTAGATCTGATA
P136	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNATCCGAGTATCGAATTCAAGCTTAGATCTGATA
P138	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCGTACACATCGAATTCAAGCTTAGATCTGATA
P213	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCTTCTCAAATCGAATTCAAGCTTAGATCTGATA
P214	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNAGGGTTTATTCGAATTCAAGCTTAGATCTGATA
P215	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGGCGTCCGTTCGAATTCAAGCTTAGATCTGATA
P216	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGTATTGTAATCGAATTCAAGCTTAGATCTGATA
P217	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCGCGCTCTTCGAATTCAAGCTTAGATCTGATA
P218	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCACTCTTCTCGAATTCAAGCTTAGATCTGATA
P219	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNTGTTTGAATTCGAATTCAAGCTTAGATCTGATA
P220	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCCTGCACACTCGAATTCAAGCTTAGATCTGATA
P221	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGATCTGCAATCGAATTCAAGCTTAGATCTGATA
P222	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNGCTTAAGGCTCGAATTCAAGCTTAGATCTGATA
P223	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCAATGTTGATCGAATTCAAGCTTAGATCTGATA
P224	CTCGGCATTCCTGCTGAACCGCTCTTCCGATCTNNNNNNNNCACCGACAGTCGAATTCAAGCTTAGATCTGATA
Primers for EVO2 cycle	
pE1	CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT
pE2	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

Table S4. Related to Figure 2. Plasmids used in this study.

Plasmids	Benchling link
pBAR1	https://benchling.com/s/U6S529CV
pBAR6	https://benchling.com/s/5bnewTrg
pBAR7	https://benchling.com/s/SZrma0HA
pBAR8	https://benchling.com/s/seq-Z0ihfMgQookfPNdrNUbi
pBAR9	https://benchling.com/s/seq-sZwuEZxGBuKkVqPVbMbH
pBAR10	https://benchling.com/s/seq-nQBMZqibosBDtnvYukeg
pBAR11	https://benchling.com/s/seq-jn3tDL5Dms6cTcYqmMjg
pBAR12	https://benchling.com/s/seq-mRqqVLrOABycUDiyT0rX
pBAR13	https://benchling.com/s/seq-h6t5XrKxsiuWnZFFlzVl
pBAR14	https://benchling.com/s/seq-spjmIEnVmMNZbO5H0ipI
pBAR15	https://benchling.com/s/seq-heABOqBRCBBygjE2t6Mx
pBAR16	https://benchling.com/s/seq-sagZuUOEY9feDxD8boY4
pBAR17	https://benchling.com/s/seq-4fPJM4Ai4Uz6JHBTovrC
pBAR18	https://benchling.com/s/seq-0Xk4ND4Cx9gKAPNcNPCF
BXL121	https://benchling.com/s/seq-TyAWiSEV1VCbCH4d57dr
BXL122	https://benchling.com/s/seq-xbtvKQuuygVIzCnmLhoX
BXL123	https://benchling.com/s/seq-dMSYSYDOLdl1J1luI62g
BXL124	https://benchling.com/s/seq-ABVeiUGaSLzIKK81GPUL
BXL147	https://benchling.com/s/seq-BxdSoqpcM2EpvtIG807E
BXL149	https://benchling.com/s/seq-eQI5JLEqROIwoJC2hOJ6
BXL160	https://benchling.com/s/seq-PlE41YUcs7dP75g8Pwyj
BXL162	https://benchling.com/s/seq-PEfY1QKeB4vsexZwKJys
BXL164	https://benchling.com/s/seq-u7Z5B54cvPoHzRJoz8Sy
BXL169	https://benchling.com/s/seq-u6rLx3wIweIUK9opIdRH
pS413 TEFpr1-linker-DHFR-HphMX	https://benchling.com/s/seq-6HsHsJUNHIooqHVxpt9i
pAG32 linker-DHFR-HphMX	https://benchling.com/s/seq-5BMVpGySmHJUiWQn7jtY

Table S5. Related to Figure 2. Yeast strains used in this study.

Name	Genotype	Source	identifier
BY4727	*MATα his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0*	ATCC	200889
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	ATCC	201388
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	ATCC	201389
SHA319	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ybr209w::GalCre-NatMX*	Jaffe et al., 2016	NA
SHA333	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-NatMX*	This paper	NA
SHA342	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-NatMX*	This paper	NA
SHA349	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ybr209w::GalCre-NatMX*	Schlecht et al., 2017	NA
XLY001	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-URA3 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY003	*MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY005	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox2272/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY009	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ybr209w::GalCre-URA3 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY011	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ybr209w::GalCre-lox2272/66-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY023	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71/66-HphMX-5'URA3-BC-loxP-lox2272/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY024	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 ybr209w::GalCre-lox2272/66-lox5171/71/66-3'URA3-BC-KanMX-lox5171 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY058	*MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 ybr209w::GalCre-lox2272/66-lox5171/71*	This paper	NA
XLY059	*MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 ybr209w::GalCre-lox71-lox2272/71*	This paper	NA
XLY065	*MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71*	This paper	NA
XLY082	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71/66 - 3'HphMX - KanMX can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY083	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71/66 - 3'NatMX - KanMX can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY085	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71/66 - 3'KanMX - HphMX can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY088	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-HphMX-loxP-5'NatMX-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY089	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-KanMX-loxP-5'HphMX-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY090	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-HphMX-loxP-5'KanMX-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY091	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71/66-HphMX-5'URA3-BC-loxP-lox5171/71 can1::MFA1pr-HIS3-MFα1pr-LEU2*	This paper	NA
XLY092	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ybr209w::GalCre-lox71-lox5171/71/66-3'URA3-BC-KanMX-lox5171 can1:: MFA1pr-HIS3-MFα1pr-*	This paper	NA

Note: All strains are S288C derivatives