Supplementary methods

Transposition assays from single copy Tf1

To monitor new transposition events occurring from the genomic copies of Tf1, an artificial intron (AI) was introduced by homologous recombination within the sequence coding for the *neo* resistance gene located within Tf1-*neo*. Briefly, strains containing Tf1 integrations #1, #2, #3 and #4 were obtained by introducing 5 to 10 μg of a fragment containing the AI purified from the plasmid pHL692 into the Tf1-containing strains YHL9629, YHL9631, YHL9632 and YHL9638 (Feng et al. 2013) along with the leu selectable plasmid pHL1288 by the lithium acetate transformation (Forsburg and Rhind 2006). Cells that allowed transformation were selected on media PM lacking leucine. The resulting colonies were then screened on media YES + G418 to identify cells that have lost their ability to grow on G418, indicating the presence of the AI within *neo*. The presence of the AI was confirmed by amplification of a 137bp-fragment between primers HL3510 and 3511.

Production of cultures with integration tags

The library of *S. pombe* containing genome-wide integration of Tf1-*neo* was constructed as previously described (Chatterjee et al. 2014), with the following modification: all incubations were carried out in PM media. Briefly, transposition was induced by expressing Tf1-*neo* from the plasmid pHL2944 in the diploid strain YHL5661 which lacked endogenous copies of Tf1. Transposition induced in media lacking vitamin B1, was then inhibited by addition of vitamin B1. The transposition frequency was monitored by transposition assay. When the transposition

frequency reached 2%, cells containing a genomic Tf1-*neo* integration and lacking the plasmid pHL2944 were selected on YES media containing FOA and G418.

Strains containing deletions of non-essential genes were purchased from the Bioneer Company (Library Version 2, http://pombe.bioneer.co.kr/).

Reconstruction of strains with integration events

We reconstructed by homologous recombination the Tf1 Integrations #5, #6, and #7, identified at the positions Chr. II position 3607268 (-), Chr. I position 678437 (-), and Chr. II position 3858489 (+), respectively. The diploid strain YHL5661 was transformed with 2 to 8 µg of fragments containing the full-length Tf1-*neo* flanked by 80 to 500bp of the target sequence, using the lithium acetate transformation. The fragment corresponding to Tf1 integrated at Chr. II position 3858489 (+) was amplified with 80bp of homology to the target site by fusion PCR using primers pairs HL3701-HL3702 and HL3703-HL3704, then HL3710-HL3711. The amplification was performed using Expand Long Range dNTPack (Roche) according to the manufacturer's recommendations. The fragments corresponding to the 2 other positions were commercially synthesized (https://www.dna20.com/ for Chr. II position 3607268 (-); Life Technologies for Chr. I 678437 (-). Fragments contained a 500bp homology to the target site, the 5' sequence of Tf1 up to the AvrII restriction site, the 3' sequence of Tf1 up to the XhoI restriction site and another 500 bp homology to the target site. The full length Tf1 flanked by target homologies was then assembled by ligating the AvrII -XhoI fragment central section of Tf1 from pHL414 into the plasmids containing partial Tf1-neo flanked by sequences of the integration site (Supplemental Table S5). The resulting plasmids containing full Tf1-*neo* flanked by sequences of the integration site (Supplemental Table S5), were digested with 10 units of SpeI

and used for lithium acetate transformation in order to achieve homologous recombination. Cells with Tf1 integrated at the loci of interest were selected onto YES containing G418. The presence of Tf1 at the expected integration site was confirmed by PCR amplification of a fragment spanning neo and the adjacent genomic region (primer pairs described in Supplemental Table S5) and by DNA blot (Suppl. Fig. S5). The resulting yeast strains are described in Supplemental Table S4.

Transposition assay

Transposition frequency was calculated according to the protocol described previously (Chatterjee et al. 2014), with the following modifications to monitor new genomic integrations of Tf1. Cultures starting at OD = 0.05 were incubated in 50 ml of PM media containing 0.2mM $CoCl_2$, 7.5mM $ZnCl_2$, 10mM caffeine or 50mM Phthalate. A solution of HCl was added at 8mM to the media containing $ZnCl_2$ and the corresponding control media for $ZnCl_2$ solubility. Cultures were diluted to OD = 5, then diluted in series of five 10-fold dilutions. The three lowest dilutions were spread onto YES plates, and the highest dilution as well as a number of cells equivalent to 25 ml of culture were spread on YES containing G418. The artificial intron allows the expression of the G418 resistance only in cells that have undergone a new genomic integration event (Levin 1995). After 3 days, the number of colonies were counted for 3 biological replicates, apart from Integration #4 in 10mM caffeine with only 2 biological replicates, and extrapolated to the number of cells in 1ml of culture. The transposition frequency corresponds to the percentage of colonies resistant to G418. The number of generations achieved during the culture was determined by measuring the OD at the beginning and end of cultures. Transposition rates were

calculating by dividing the transposition frequency by the number of generations. The p-values were calculated using Student's t-test.

References

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