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Supplemental Information

Dense Transposon Integration Reveals Essential Cleavage and Polyadenylation Factors Promote Heterochromatin Formation

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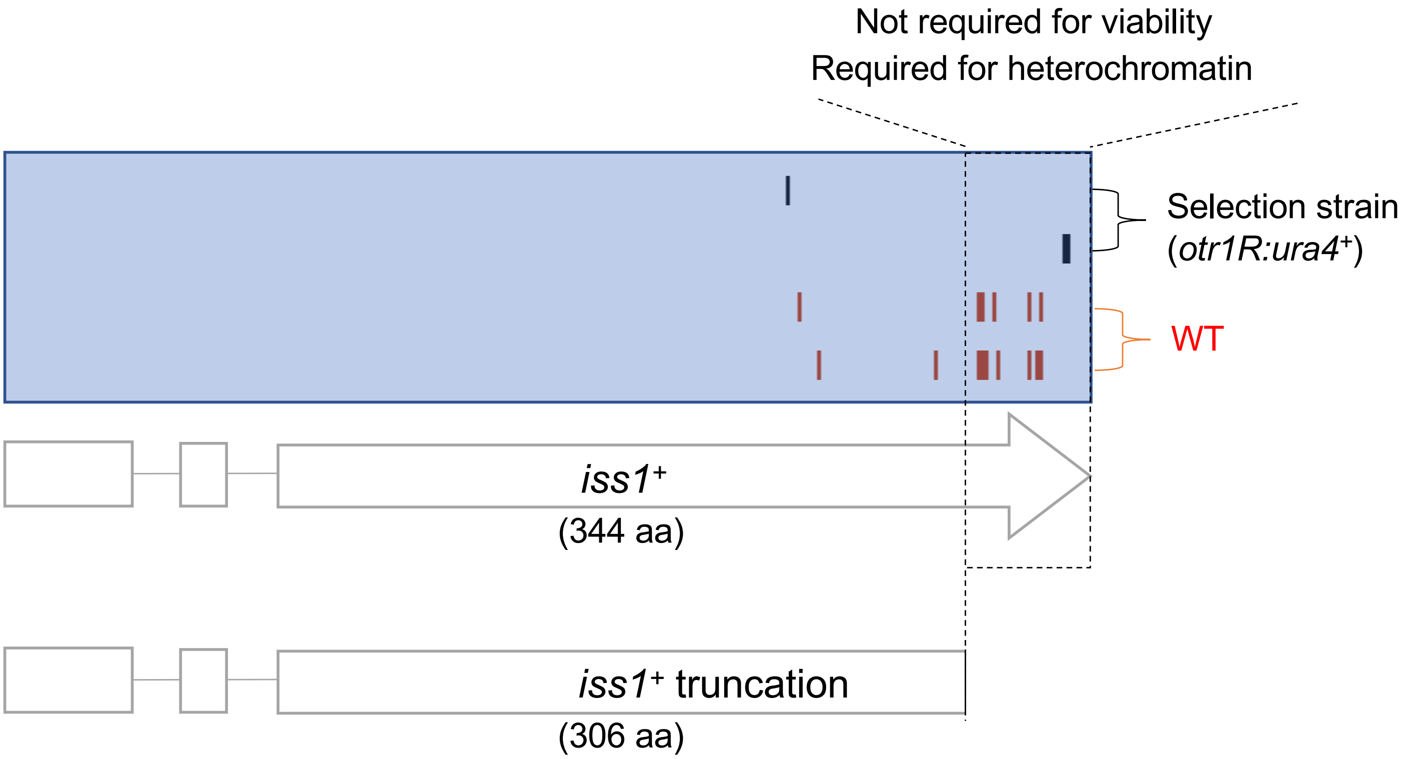


Figure S1. Hermes integration patterns from 80 generation cultures showing that inserts are tolerated in the C terminus in the wild type strain but not in the strain with the otr1R:ura4+ reporter, related to Fig. 2B.

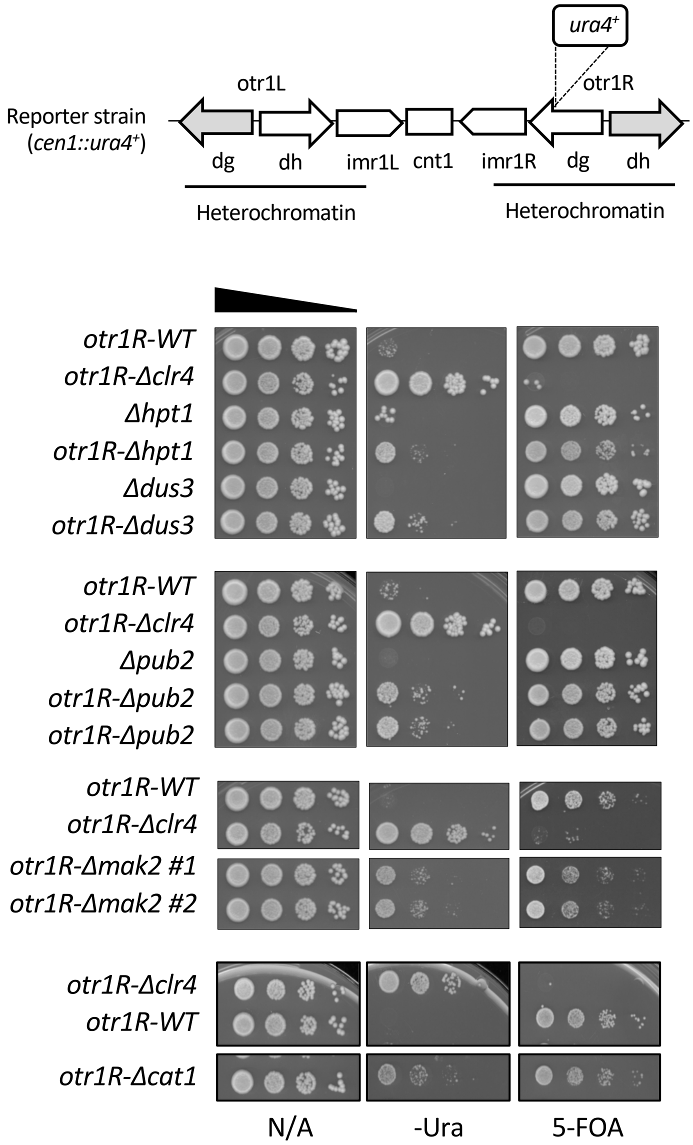


Figure S2. Candidate heterochromatin factors contribute to silencing of *ura4* at the outer centromere repeat otr1R, related to Fig. 1A. Candidate heterochromatin genes were deleted in strains with the *ura4*::otr1R reporter. Serial dilution of strains placed on plates lacking uracil (-Ura) or containing 5-FOA. Deletion of candidate genes resulted in increased growth on –Ura and in some cases reduced growth on 5-FOA.

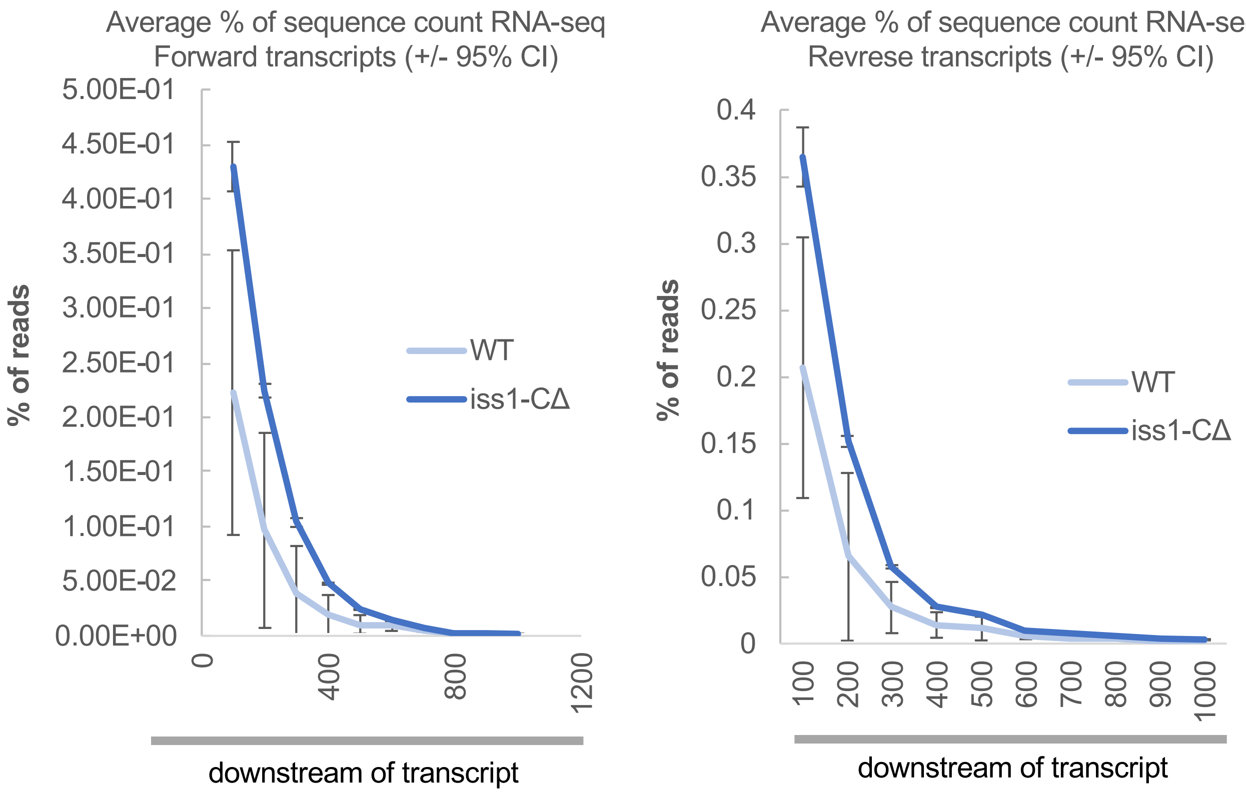


Figure S3. Average % of sequence counts by RNA-seq in three biological replicate libraries of WT or iss1-ΔC, related to Fig. 4C. The area included within the 95% CI (confidence interval) of WT is outside of the 95% CI of iss1-CΔ in the 3' region after the transcript.

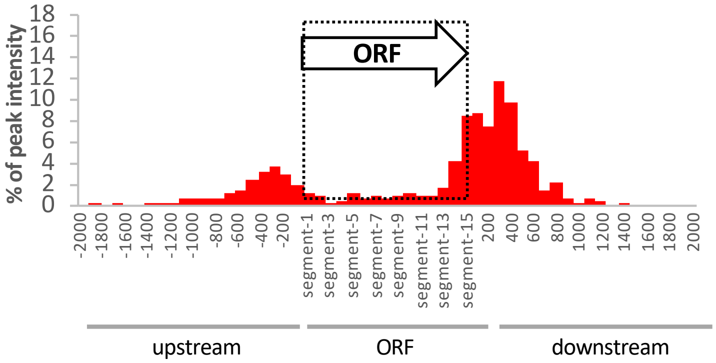
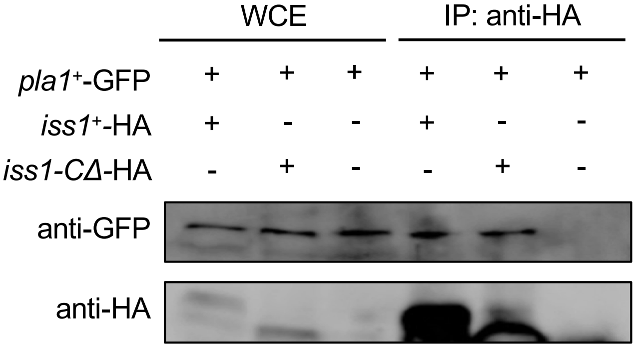


Figure S4. Iss1 binding by ChIPseq clustered 400 bp downstream of coding sequences of genes, related to Fig. 6B. A modest amount of Iss1 also clustered upstream of ORFs but this is greatly reduced if binding to tandem intergenic sequences is not included (Fig. 6B).

A. Co-immunoprecipitation of Iss1 and Iss1-ΔC associated with Pla1.



B. Co-immunoprecipitation of Iss1 and Iss1-ΔC associated with Rrp6.

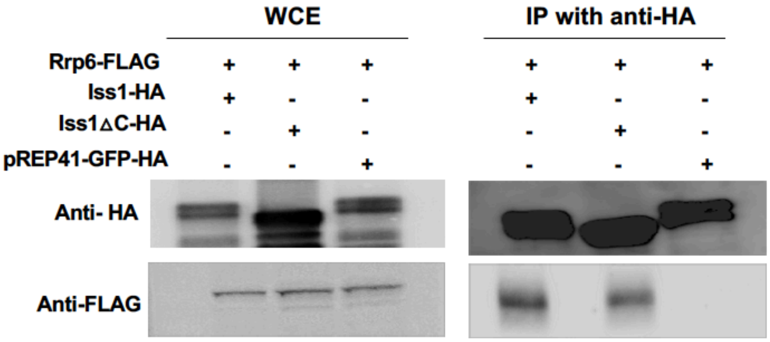


Figure S5. Co-immunoprecipitation of Iss1 and Iss1-ΔC associated with Pla1 and Rrp6, related to Fig. 5D. A. Co-immunoprecipitation of Iss1 and Iss1-ΔC associated with Pla1. the *S. pombe* strain YHL12284 (h90 pla1-GFP-kanR, ade6-M216, leu1) was transformed with plasmids expressing Iss1-3HA or Iss1-ΔC-3HA (lacking the C-terminal 39 amino acids). B. Co-immunoprecipitation of Iss1 and Iss1-ΔC associated with Rrp6. the *S. pombe* strain YHL12284 (h90 pla1-GFP-kanR, ade6-M216, leu1) was transformed with plasmids expressing Iss1-3HA or Iss1-ΔC-3HA (lacking the C-terminal 39 amino acids).

All sample Differential transcript (n=1,056) cov-based PCA Scatterplot

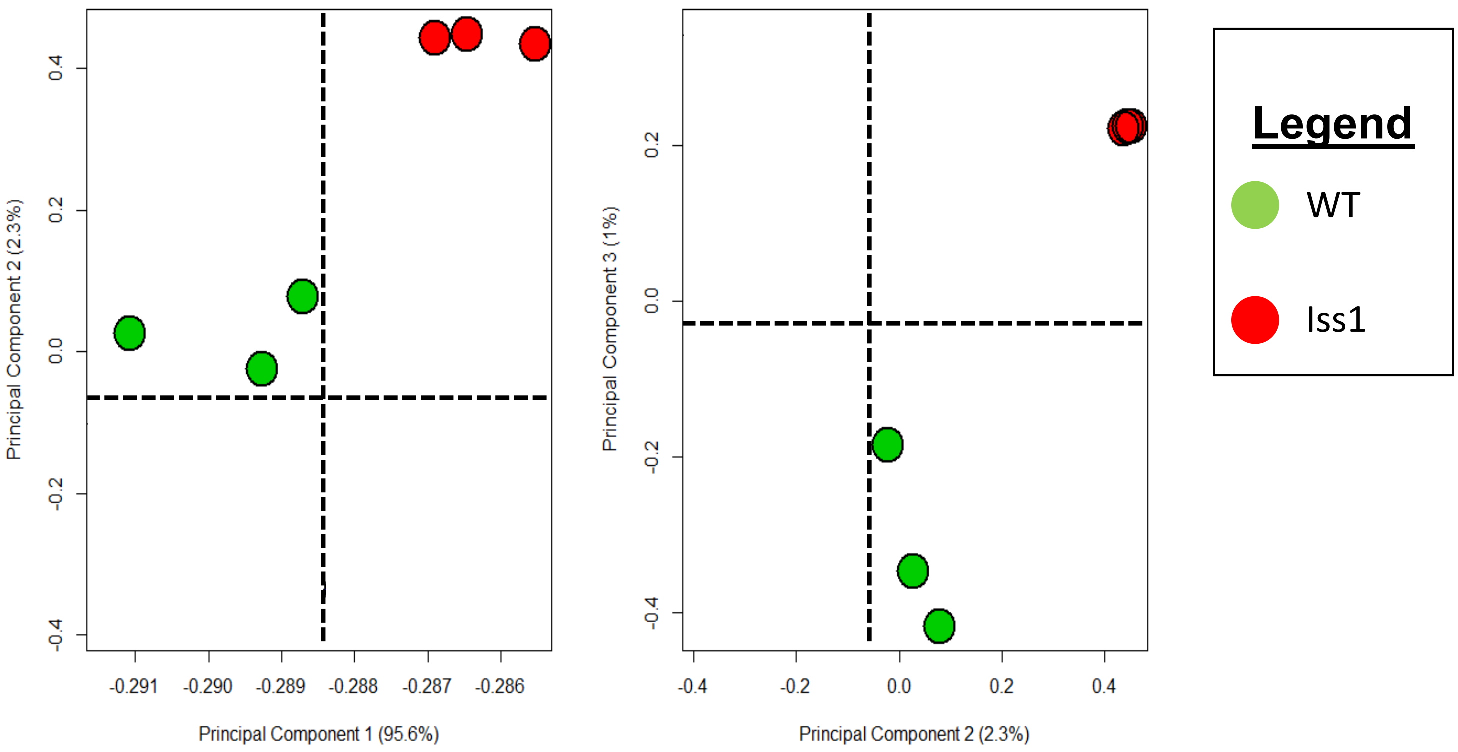


Figure S6. Covariance-based PCA scatter plot of the RNA expression enumerated per sample, related to Star Methods RNAseq analysis.

Supplementary Table S1. Total numbers of unique Hermes insertions in independent libraries, related to Fig. 1A.

	WT-cen1 (G=5)		cen1::ura4 (G=5)		WT-cen1 (G=80)		cen1::ura4 (G=80)	
Replica	A	B	A	B	A	B	A	B
Unique insertions	426,000	546,000	842,000	608,000	303,000	332,000	422,000	353,000
Average insertion density	1 insert per 30 bp	1 insert per 23 bp	1 insert per 15 bp	1 insert per 21 bp	1 insert per 42 bp	1 insert per 38 bp	1 insert per 30 bp	1 insert per 36 bp

Strains:

WT-cen1 (Wild Type centromere 1)

YHL9451A (replica A) and YHL9451C (Replica B) are independent transformants of (YHL912) h- ura4-294 leu1-32/ pHL2577 and pHL2578

cen1::ura4 (reporter strain)

YHL10160B (replica A) and YHL10161B (replica B) are independent transformants of (Spr156) Mat/Msmt0; Leu1-32; M210; His2; Ura4 DS/E; Otr1R(Sph1)::ura+/ pHL2577 and pHL2578

Abbreviations

G=5; 5 generations on FOA

G=80; 80 generations on FOA

Supplementary Table S4. 15 genes upregulated by *iss1-ΔC* and *rrp6Δ*, related to Fig. 4G.

Gene	**Description**	**i*ss1-CΔ* vs WT (2> fold)**	**Rrp6 targets (Mukherjee et al.,2016)**
fio1	*plasma membrane iron transport multicopper oxidase*	6.83	13.25
fip1	*plasma membrane iron permease*	5.09	2.46
str1	*plasma membrane siderophore-iron transmembrane transporter*	4.86	3.97
psf3-antisense-1	*ncRNA*	4.59	6.55
SPBPB21E7.04c	*O-methyltransferase, human COMT catechol homolog 2*	3.97	7.46
meu19	*ncRNA*	3.76	14.27
SPNCRNA.1696	*lncRNA nam3*	3.36	9.15
SPNCRNA.1444	*ncRNA*	2.91	14.25
SPNCRNA.893	*ncRNA*	2.61	26.36
SPAC890.05-antisense-1	*ncRNA*	2.58	4.21
srx1	*sulfiredoxin*	2.28	5.99
SPNCRNA.723	*snoRNA*	2.15	17.26
sme2	*meiRNA*	2.05	260.41
SPBC660.06	*WW domain containing conserved fungal protein*	2.03	2.81
SPBC1711.15c	*S. pombe specific protein*	2.00	10.07

Supplementary Table S6. Enrichment of Iss1 bound genes in three independent studies of genes regulated by RNA elimination, related to Star Methods section: Overlap of gene sets independently found to be involved in RNA elimination.

Genes regulated by RNA elimination	Number of genes with iss1 peak that are regulated by RNA elimination	Total number of genes with iss1 peak (p<0.05)	Fold enrichment	Total number of genes regulated by RNA elimination	Number of genes in genome	P-value (hypergeometric distance)	FDR: False Rate Discovery, p-value
Genes within heterochromatin islands (*Zofall M et al., 2012*)	10	1002	1.89953426	36	6852	0.029687127	0.02968713
RNAs bound by Mmi1 (*Touat-Todeschini L et al., 2017***)**	13	1002	2.96327345	30	6852	0.000137131	0.000137131
Genes suppressed by Rrp6 (*Mukherjee K et al., 2016***)**	49	1002	1.28382316	261	6852	0.035561795	0.0355618

Supplementary Table S7. Media composition for integration profile experiments, related to Star Methods section: Hermes integration profiles.

Media	FOA	Leu	Ura	Thiamine	G418
YES	-	-	-	-	-
YES + G418	-	-	-	-	500 μg /ml
PMG + U –L +B1 +G418	-	-	50 μg /ml	10 μM	500 μg /ml
PMG +U – L – B1 +G418	-	-	50 μg /ml	-	500 μg /ml
PM + FOA	1 mg/ ml	250 μg /ml	50 μg /ml	10 μM	-
PMG + FOA + G418	1 mg/ ml	250 μg /ml	50 μg /ml	10 μM	500 μg /ml