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Supplemental Information

Inheritance of a Phenotypically Neutral Epimutation Evokes Gene Silencing in Later Generations

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Transgenerational inheritance of an RNAi-directed gene silencing phenotype in the absence of the original siRNA trigger, Related to Figure 1

A, Schematic diagram depicting euchromatic origin (green) and target (red) of synthetic *ade6-hp* siRNAs. Gene silencing is not initiated in the presence of wild type Paf1C (Kowalik et al., 2015; Yu et al., 2018). **B**, Genotypes of strains used to assess transgenerational inheritance of gene silencing in *paf1-Q264Stop* cells. **C**, Representative pedigree illustrating spore viability and inheritance of the red silencing phenotype over 18 generations. Red *paf1-Q264Stop* (*ade6*$^{+}$ OFF) colonies were repeatedly crossed with white *paf1-Q264Stop* (*ade6*$^{+}$ ON) cells and tetrads were dissected on YE plates. Note that the ade6-hairpin, which initiated silencing in the parental strain (F0), was no longer present from F1 onwards. Each row represents the four spores that were micro-dissected from one tetrad. Grey dotted circles indicate spores that were dissected but failed to form a mitotically growing colony. Yellow box highlights F10 spores that gave rise to cells that were analyzed in Figure 1. **D**, Quantification of spore viability, as well as inheritance and mitotic stability (maintenance) of the *ade6*$^{+}$ silencing phenotype for five generations of three independent pedigrees. **E**, Representative photograph of a spore dissection experiment on YE plates. Left picture shows generation F8 of pedigree 2 as illustrated in c (homozygous *paf1-Q264Stop* cross). Right picture shows a heterozygous cross of red *paf1-Q264Stop* (F7) and white *paf1*$^{+}$ cells. The + symbol denotes those spores that inherited the wild type *paf1*+ allele. Note that the spore viability phenotype observed in homozygous crosses was largely rescued in the heterozygous crosses.

Figure S2. Secondary *ade6*$^{+}$ siRNAs persist in wild type progeny if they were present in the previous *paf1*$^{+}$ mutant generation, Related to Figure 2

A and B, Cells originating from four independent white (A, *ade6*$^{+}$ ON) and red (B, *ade6*^{si3} OFF) *paf1-Q264Stop* spores of generation 10 were crossed with wild type cells. *paf1*$^{+}$ progeny was subjected to sRNA sequencing. sRNA profiles over the *ade6*$^{+}$ gene of cells carrying either *ade6*$^{+}$ ON (A) or *ade6*^{si3} ON (B) alleles are shown. **C**, Length distribution and 5' U bias of small RNAs shown in B, which are characteristic of siRNAs. A-C, Read counts were normalized to library size.

Figure S3. The *ade6*$^{+}$ silencing phenotype is lost in wild type cells, but is re-established by repeated Paf1C impairment, Related to Figures 2 and 3

A, Crossing scheme for testing the inheritance of the marked *ade6*^{si3} epiallele in wild type cells and re-establishment of the silencing phenotype upon repeated Paf1C impairment. **B**, Representative images of a typical experiment performed to follow the *ade6*$^{+}$ silencing phenotype across generations (*paf1-Q264Stop* to *paf1*$^{+}$ to *paf1-Q264Stop*). RSA, random spore analysis. **C**, Recurrence frequency of the *ade6*$^{+}$ silencing phenotype (red color) in *paf1-Q264Stop* progeny whose parents were phenotypically normal (white). n=23 different crosses over 3 generations as depicted in a. At least 500 colonies (spores) per cross were scored for the silencing phenotype. Centre values denote the mean; error bars denote s.d.; P values were calculated with two-tailed Student's t-test **D,** Mitotic stability of the silent state that was re-established upon repeated *paf1*$^{+}$ mutation. 3 red *paf1-Q264Stop* F12 colonies for each of the 4 crosses analyzed in c were investigated (n=12). At least 500 cells per F12 clone were plated at single cell density and inspected for the silencing phenotype after mitotic propagation. The silencing phenotype was never observed if the original F10 colony was white (n>1500 colonies per plating). Centre values denote the mean; error bars denote s.d.; P values were calculated

with two-tailed Student's t-test. **E**, UCSC genome browser shots showing the ChIP-seq profiles at *ade6*$^{+}$ for H3K36me3, total H3, and input for wild type (with and without marked *ade6*$^{+}$) and *paf1-Q264Stop* cells. Profiles were normalized to library size. n = 3 different crosses.

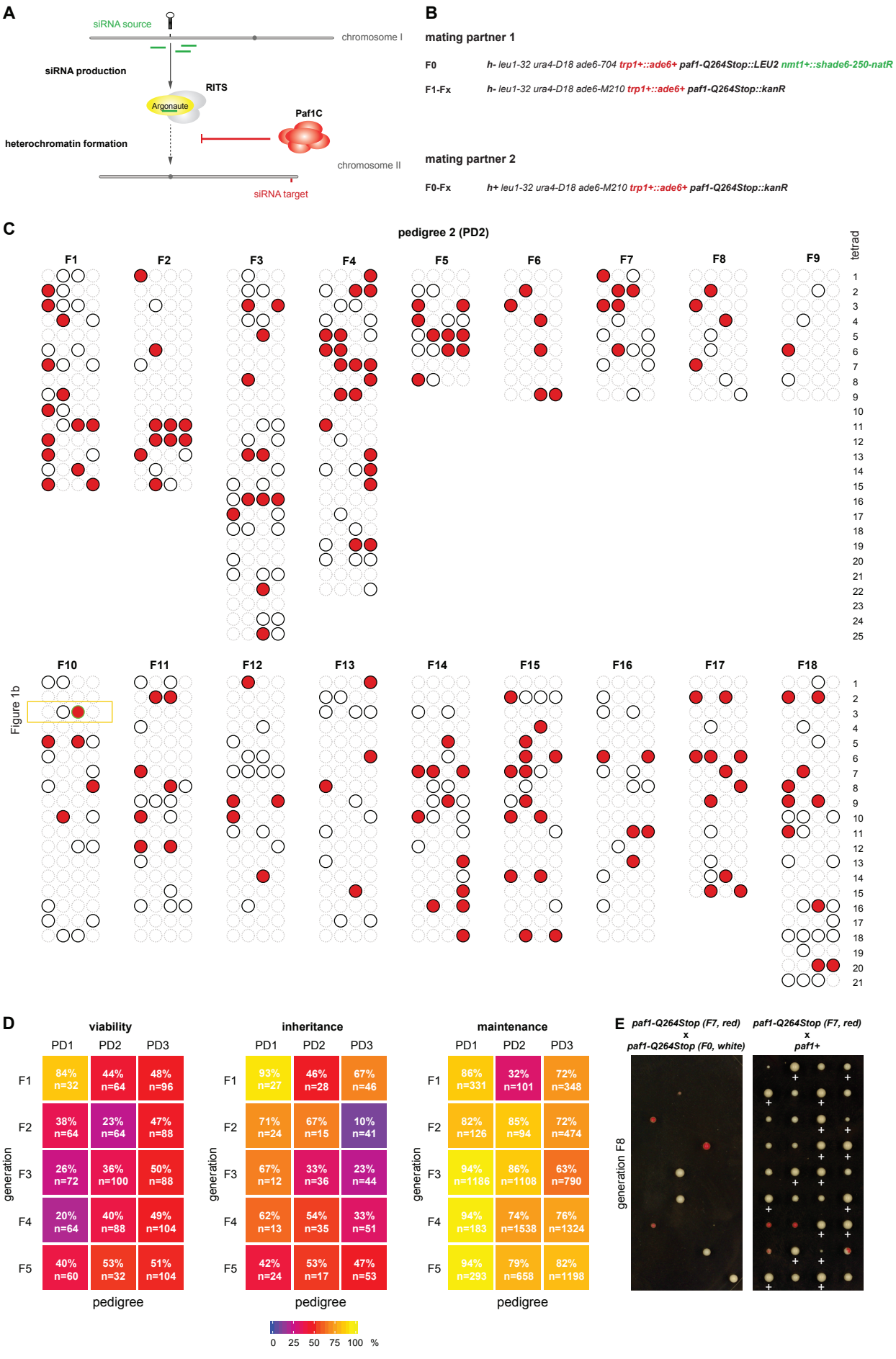
Figure S4. H3K9 tri-methylation activity of Clr4 is required for continuous *ade6*$^{+}$ siRNA generation, Related to Figure 4

A, Red *paf1-Q264Stop* colonies (F10) were crossed with *paf1*$^{+}$ cells either lacking key RNAi factors (Ago1, Dcr1, or Rdp1) or the H3K9 methyltransferase Clr4, or expressing a mutant Clr4 that is deficient in catalyzing H3K9me3 (Clr4-F449Y). 3 spores for each combination of *paf1*$^{+}$ with one of the RNAi, Clr4 mutant alleles or wild type (*ade6*^{si3}) were expanded and subjected to small RNA sequencing. **B and C**, siRNA profiles over *ade6*$^{+}$ and the *dg* and *dh* repeats of *otrIR* are shown. Counts were normalized to library size.

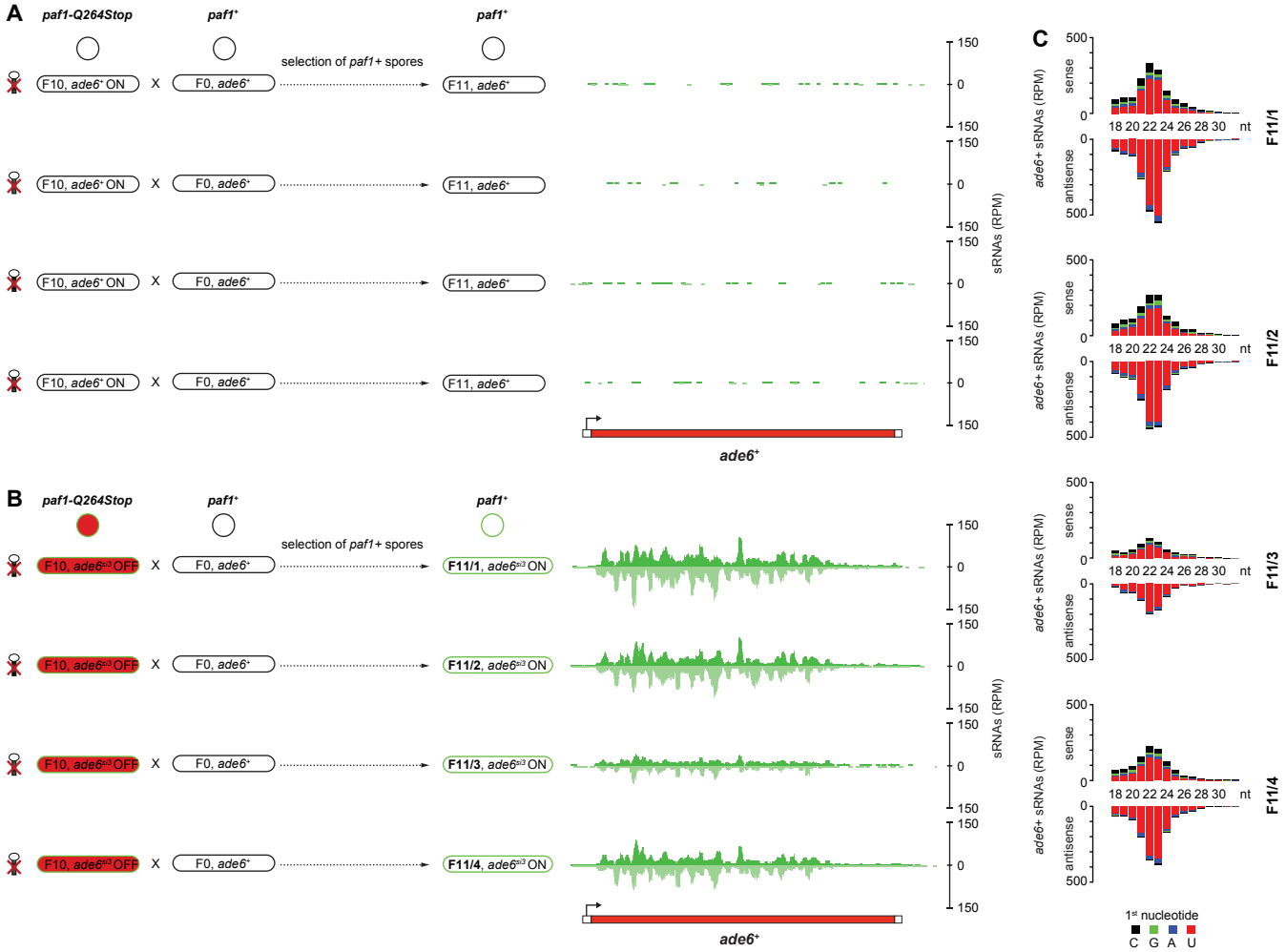
Figure S5. Impaired Paf1C activity leads to the production of endogenous siRNAs that are complementary to protein-coding genes, Related to Figure 1

A, Wild type and different Paf1C mutant cells were subjected to small RNA sequencing. (A-C) All three *S. pombe* chromosomes are shown. siRNA peaks mapping to protein-coding genes are indicated by the black or green dots. Only small RNA reads with a length distribution and 5' U bias characteristic of siRNAs were considered. Gene names are indicated on top. **B**, Parental *paf1-Q264Stop* cells (indicated by a red line in A) were crossed and their descendants of the fourth generation were subjected to small RNA sequencing. Two independent crosses were analyzed (pedigree 1 and 2). Black dots denote siRNA peaks that arose newly over the four generations. Green dots denote siRNA peaks that were already present in the parental cells. **C**, F1 spores of another cross (pedigree 4) of the same parental cells as in B (indicated by a red line in A). Green dots denote siRNA peaks that were already present in the parental

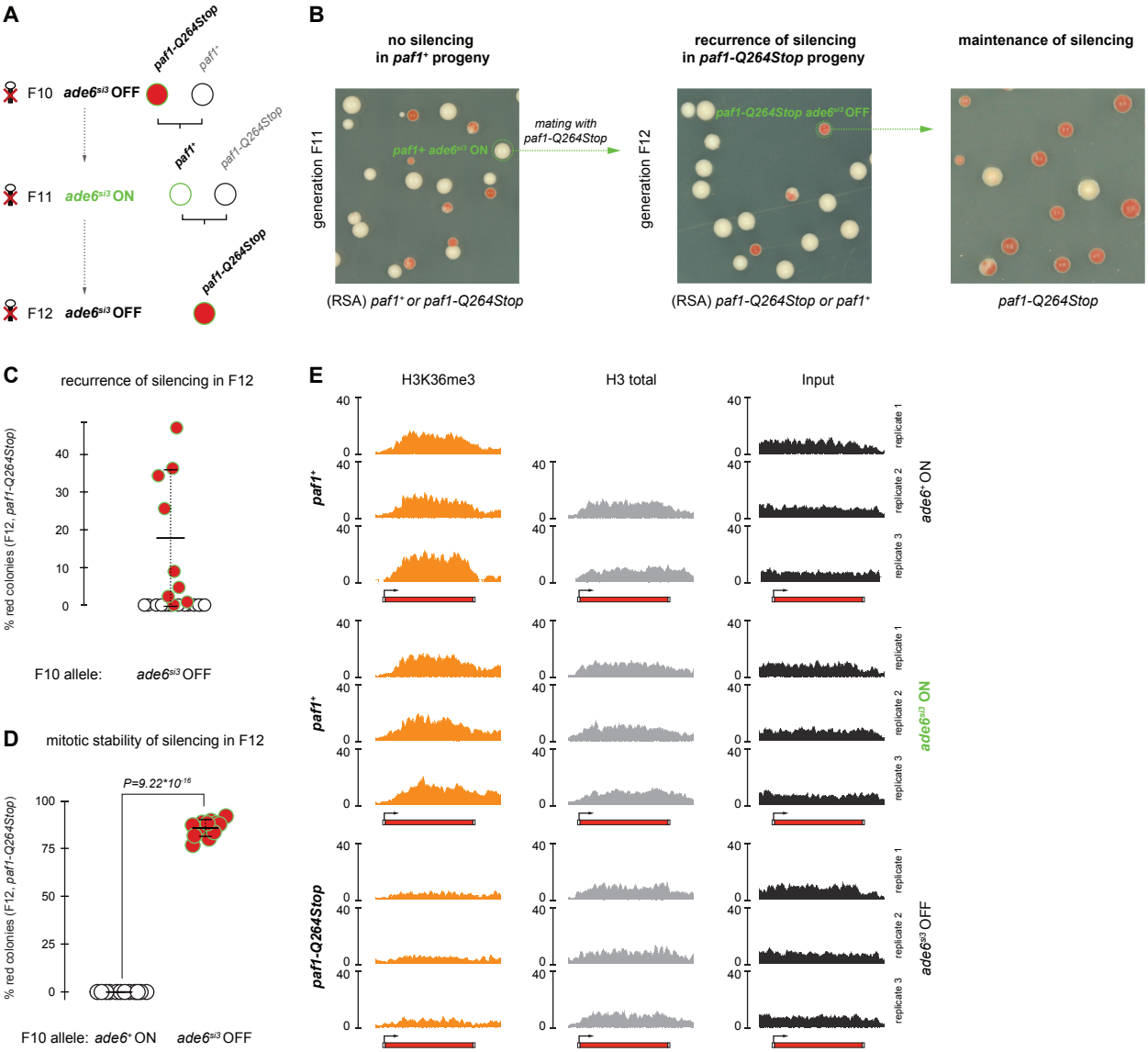
cells. Blue dots denote siRNAs that were absent in parental cells but arose also in other pedigrees.



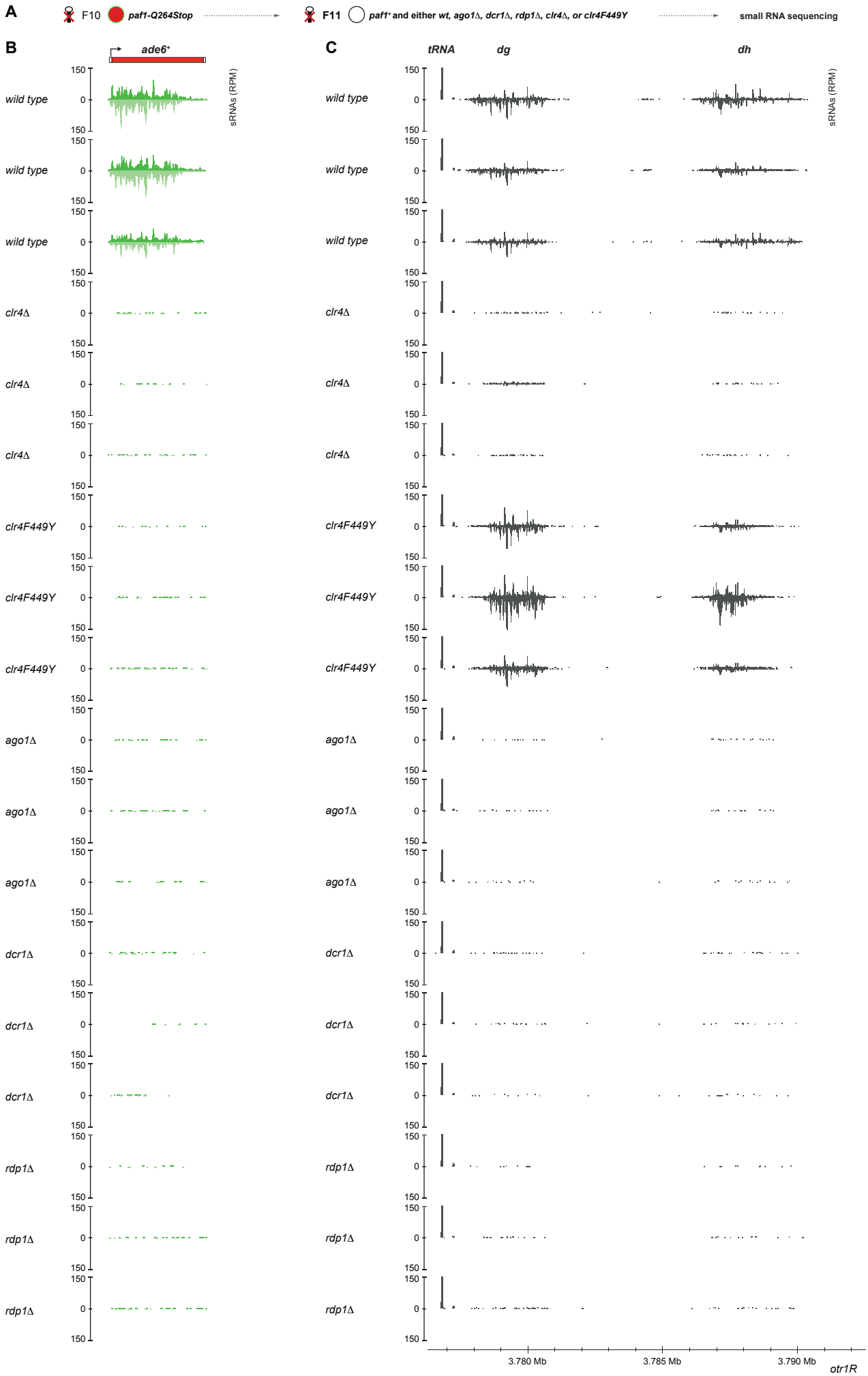
Dümpelmann *et al*, Figure S1



Dümpelmann *et al*, Figure S2



Dümpelmann *et al*, Figure S3



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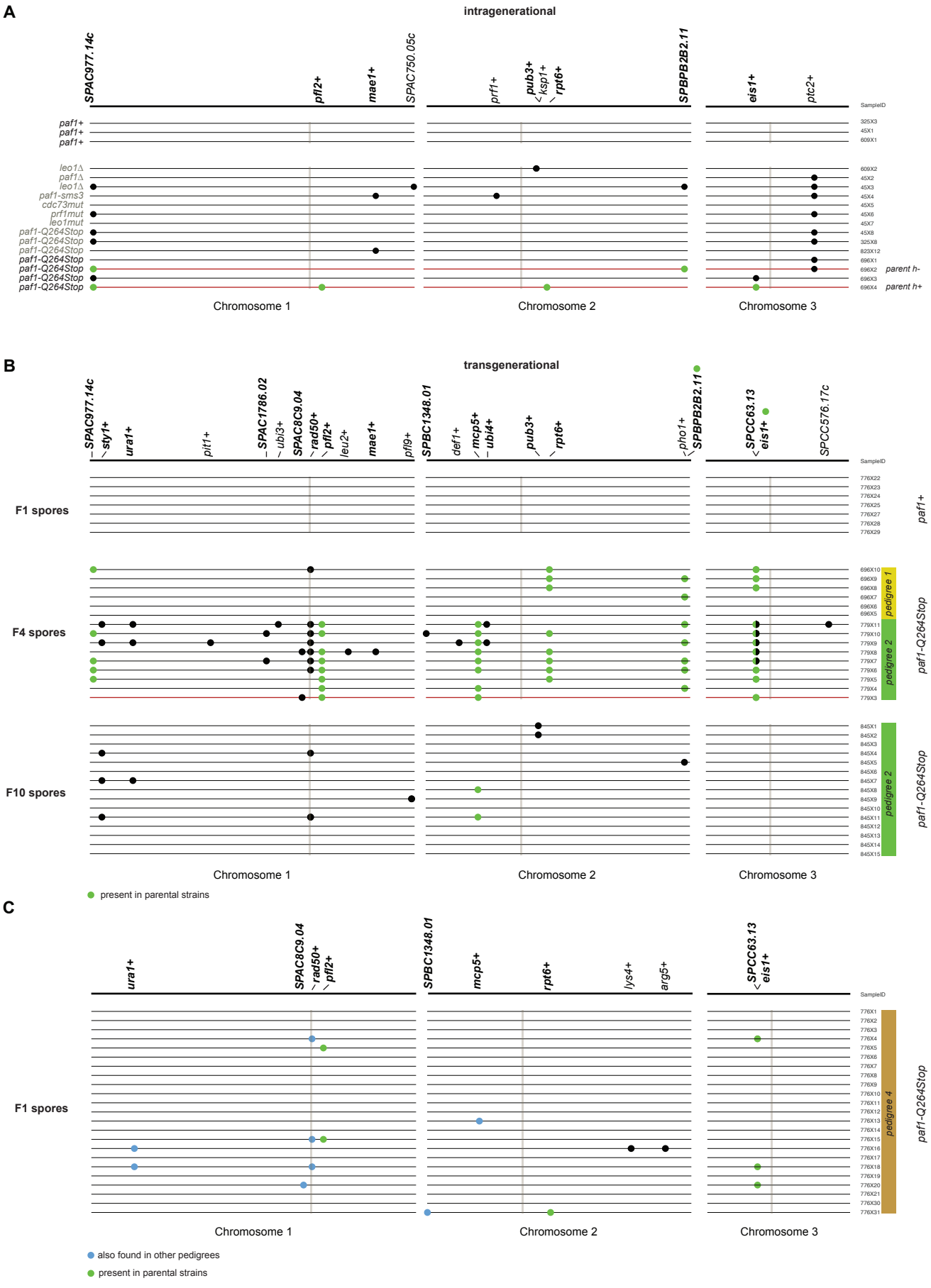

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