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## RESEARCH ARTICLE OPEN ACCESS

# The 5-Fluorouracil RNA Expression Viewer (5-FU<sup>R</sup>) Facilitates Interpreting the Effects of Drug Treatment and *RRP6* Deletion on the Transcriptional Landscape in Yeast

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**Keywords:** 5-Fluorouracil | dsRNA | lncRNA | meiosis | mitosis | RRP6

## ABSTRACT

*Saccharomyces cerevisiae* is an excellent model to study the effect of external cues on cell division and stress response. 5-Fluorouracil (5-FU) has been used to treat solid tumors since several decades. The drug was initially designed to interfere with DNA replication but was later found to exert its antiproliferative effect also via RNA-dependent processes. Since 5-FU inhibits the activity of the 3′–5′-exoribonuclease Rrp6 in yeast and mammals, earlier work has compared the effect of 5-FU treatment and *RRP6* deletion at the transcriptome level in diploid synchronized yeast cells. To facilitate interpreting the expression data we have developed an improved 5-Fluorouracil RNA (5-FU<sup>R</sup>) expression viewer. Users can access information via genome coordinates and systematic or standard names for mRNAs and Xrn1-dependent-, stable-, cryptic-, and meiotic unannotated transcripts (XUTs, SUTs, CUTs, and MUTs). Normalized log<sub>2</sub>-transformed or linear data can be displayed as filled diagrams, line graphs or color-coded heatmaps. The expression data are useful for researchers interested in processes such as cell cycle regulation, mitotic repression of meiotic genes, the effect of 5-FU treatment and Rrp6 deficiency on the transcriptome and expression profiles of sense/antisense loci that encode overlapping transcripts. The viewer is accessible at <http://5fur.genouest.org>.

## 1 | Introduction

The budding yeast *Saccharomyces cerevisiae* is a eukaryotic model organism useful for studying the transcriptional response and resistance to antiproliferative drugs, such as 5-fluorouracil (5-FU), which has been widely used to treat solid tumors for several decades (Carlsson, Hu, and Ronne 2018; Fang, Hoskins, and

Butler 2004; B. Xie et al. 2019). 5-FU was initially designed to be a DNA replication inhibitor, but the drug was subsequently found to also interfere with RNA-dependent processes (Hoskins and Scott Butler 2007); for review, see (Chalabi-Dchar et al. 2021).

The yeast genome comprises *bona fide* open reading frames (ORFs) that encode mRNAs translated into biologically active

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**Take away**

- We present an improved 5-Fluorouracil RNA (5-FU<sup>R</sup>) expression viewer
- Strand-specific RNA-Seq data are available for mRNAs and different classes of lncRNAs
- Normalized data are displayed as filled diagrams, line graphs or heatmaps
- The expression data are pertinent for regulation of mitotic and meiotic genes
- Users can interpret the expression profiles of sense/antisense transcripts

proteins, so-called dubious ORFs that typically overlap protein coding sequences and a wide variety of noncoding genes (Goffeau et al. 1996). The latter are annotated based on their RNA's stability in mitosis (stable unannotated transcripts, SUTs [Xu et al. 2009]), meiosis (meiotic unannotated transcripts, MUTs [Lardenois et al. 2011]), a mutant lacking the 3'-5' exoribonuclease Rrp6 (cryptic unannotated transcripts, CUTs [Neil et al. 2009; Xu et al. 2009]) or a strain deficient for the 5'-3' exoribonuclease Xrn1 (Xrn1-sensitive unstable transcripts, XUTs [van Dijk et al. 2011]).

Expression viewers are useful tools provided by the bioinformatics community for biomedical researchers, who lack programming skills but wish to interpret genomics data. Several viewers are available for budding and fission yeast GeneChip expression data (<http://bahlerweb.cs.ucl.ac.uk/cgi-bin/SPGE/geexview> [Mata et al. 2002; Rustici et al. 2004]; [www.germonline.org](http://www.germonline.org) [Lardenois et al. 2010]) and tiling array data that cover the entire genome on both strands (<http://sgv.genouest.org> [Lardenois et al. 2011]). Other data sources cover more recent RNA-Sequencing data obtained with wild-type and *xrn1* mutant *S. cerevisiae* strains in different backgrounds ([http://vm-gb.curie.fr/mw2/XUT\\_catalog/](http://vm-gb.curie.fr/mw2/XUT_catalog/); [van Dijk et al. 2011]), different phases of the *S. pombe* life cycle (<http://vm-gb.curie.fr/mw1/>; [Wery et al. 2018]) or wild-type and various RNA processing mutants in *N. castellii* (<http://vm-gb.curie.fr/castellii/>; [Szachnowski et al. 2019]). Finally, specialized online tools provide access to profiling data for stable sense/antisense double-stranded RNA (dsRNA) formation in haploid wild-type and mutant *S. cerevisiae* strains and diploid cells undergoing mitotic division or meiotic development ([http://vm-gb.curie.fr/mw2/small\\_RNA-seq/](http://vm-gb.curie.fr/mw2/small_RNA-seq/) [Wery et al. 2016]; <http://sensr.genouest.org>, Szachnowski et al. submitted).

5FU<sup>R</sup> provides access to strand-specific RNA-Sequencing data obtained with synchronized diploid wild-type cells in the absence and presence of 5-FU and *rrp6* mutant yeast cells. We describe the user interface, summarize data output options and show examples of genes peaking during the mitotic cell cycle and meiotic development and the stability of different types of noncoding RNAs under various conditions. The tool allows for building hypotheses about cell cycle regulation during mitotic division, mitotic repression of meiosis-specific genes, the potential of sense/antisense loci encoding overlapping transcripts to form stable dsRNAs and the cellular stress response to 5-FU treatment versus *RRP6* deletion.

**2 | Materials and Methods****2.1 | Database Development**

5-FU<sup>R</sup> is based on Illumina RNA-sequencing data in bigWig format and locally stored genome annotation data in gtf format. 5-FU<sup>R</sup> runs in an open-source Apache web server environment with the Ubuntu Linux operating system. The graphical user interface (GUI) is generated by a script written in PHP. A Python script generates the JavaScript Object Notation (JSON) object containing image information. The *Plotly* JavaScript graphing library employs the JSON object to generate the image displayed in the dynamic report page.

**2.2 | Yeast Strains and Media**

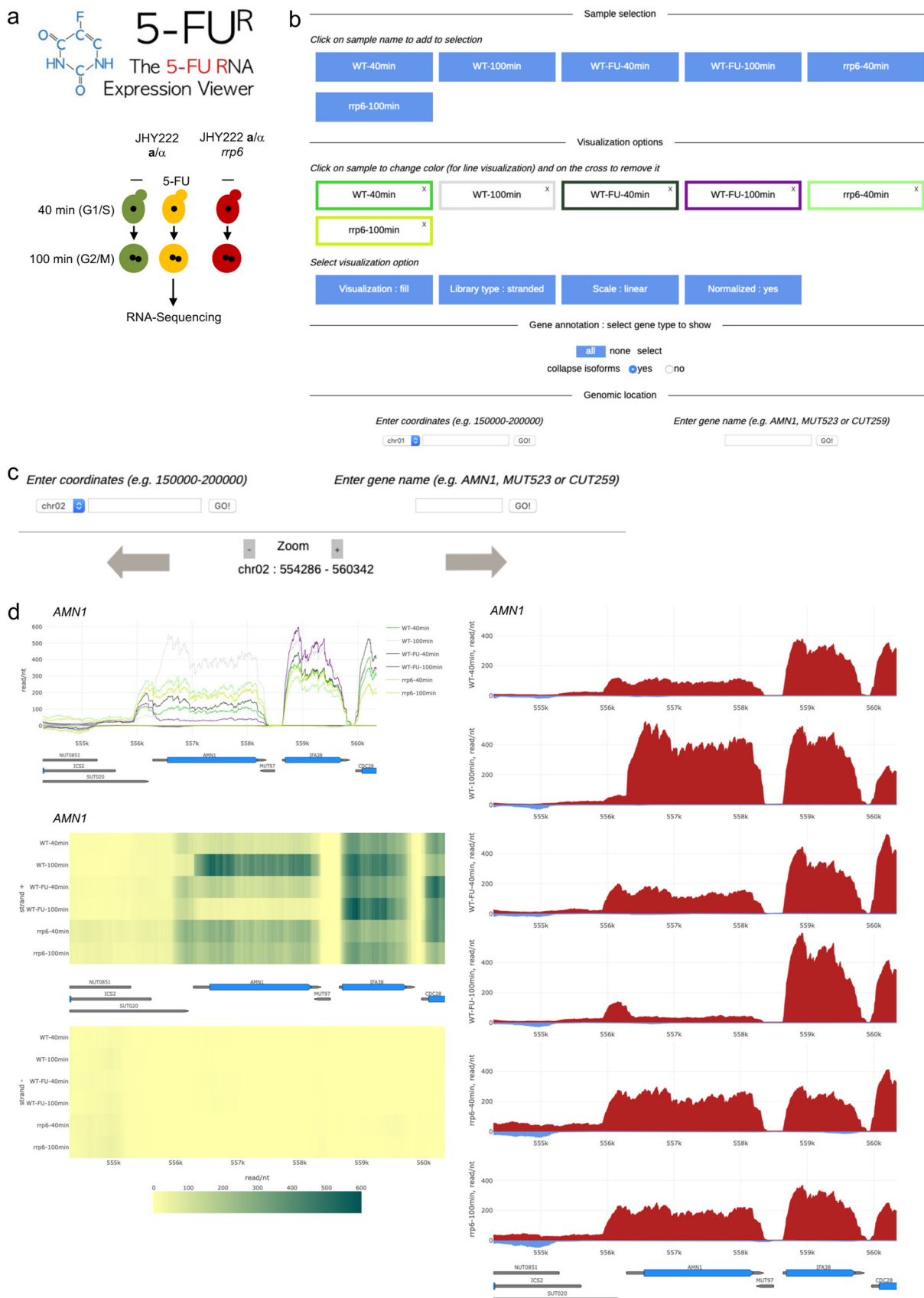
The strains, culture conditions and RNA-sequencing data production are described in detail in reference (B. Xie et al. 2019). Briefly, we cultured diploid JHY222 wild type and *rrp6* deletion strains in rich medium (YPD) until they reached saturation. Quiescent wildtype and *rrp6* mutant cells were washed and released into fresh YPD and compared to wildtype cells cultured in YPD supplemented with 5-FU and harvested at time points corresponding to G1/S and G2/M phases.

**2.3 | Relevant RNA Profiling Data**

dsRNA profiling data for diploid cells in mitosis and meiosis are accessible at <https://sensr.genouest.org> (Szachnowski et al. submitted). Normalized expression data for mitotic, meiotic and starving cells obtained with yeast tiling arrays (GeneChips covering top and bottom strands of the yeast genome) are available via <http://sgv.genouest.org> (Lardenois et al. 2011).

**3 | Results****3.1 | The 5-FU<sup>R</sup> Query Interface**

The data were generated with samples from starvation-arrested wildtype yeast cells harvested 40 and 100 min after having been released into rich medium in the presence and absence of 5-FU; untreated *rrp6* mutant cells were used as controls (Figure 1a). Users access data by pressing *Start* in the welcome page and selecting (or removing) single or averaged (merged) samples from popup menus (Figure 1b). The popup menus in the section *Visualization options* include different output graphs (heatmap, fill, line), the library type (stranded/not stranded), the scale (linear or log<sub>2</sub>) and the possibility to display normalized data (yes, no). Users can also adjust the report page's content by selecting items in the *Gene annotation* section; it is possible to display all, none or only selected noncoding and protein-coding RNAs and RNA isoforms. Finally, users can home in on specific regions or individual genes of interest via the *Genomic location* section by selecting chromosomes and providing base coordinates (e.g., chr01 and 1–50,000), or by entering systematic or standard gene names corresponding to coding or noncoding transcripts into the text field. The report page includes a text



**FIGURE 1** | 5-FUR<sup>R</sup> query options and data display. (a) A schematic shows the logo and the experimental approach used for data production. (b) The interface for selecting samples, display options and annotation data is shown. (c) Query, zoom and up/downstream shift options are shown. (d) Different types of linear data displays are given using *AMN1* as an example.

field for genome coordinates (top left) or individual genes (top right), arrows for moving the display window up- or downstream of the initial location and a zoom option (Figure 1c).

### 3.2 | The 5-FU<sup>R</sup> Report Page Content, Data Navigation and Browser Compatibility

The graphical options comprise color-coded or filled line diagrams and heatmaps that allow for flexible linear/log<sub>2</sub>-transformed data display depending on the target gene's differential expression patterns. Genome coordinates (*x*-axis) are plotted against the RNA levels (read/nucleotide; *y*-axis) for individual or selected samples (see e.g., *AMNI* in Figure 1d). Mousing over the graph displays a toolbox (top right corner) to download an image of the data in the png format. Entering the coordinates of entire chromosomes provides a global view and allows for homing in on loci that show signals indicating strong differential expression by narrowing down the corresponding genome coordinates (Figure 2).

We note that in the *linear* data view extremely high values can render weaker neighboring signals barely visible. This issue can be circumvented by using the zoom function, adjusted genome coordinates that exclude strong signals or the *log<sub>2</sub>* data view. We tested 5-FU<sup>R</sup> using the most popular browser software running under Windows and macOS operating systems. Please note that macOS Safari/Chrome users must enter the initial query by pressing the GO button rather than the return key to access the report page, otherwise Safari (but not Chrome) adds an additional sample rather than providing a data output for the selected samples.

### 3.3 | Cell Cycle Regulation and Function

Comparing drug treatments in populations of asynchronously growing haploid cells is a common practice; however, in the case of a compound that triggers a cell cycle arrest the drug response pattern may overlap with normal transcript level fluctuations as cells progress through mitotic divisions. The data provided via 5-FU<sup>R</sup> were therefore obtained with synchronized wild-type cells that were arrested by starvation and then released into rich medium with or without 5-FU at time points that broadly correspond to G1/S (40 min after release) and G2/M (100 min after release). This design detects transcripts that display stage-specific peak levels, such as the G1 and G2 cyclins *CLN1* and *CLB1* (Figure 3a). It is also possible to compare the effects of 5-FU and *RRP6* deletion on cell cycle-regulated genes; for example, *CDC19*, which plays a role in glycolysis (Figure 3b) (Sprague 1977) and *BUD9*, which is important for bud site selection (Figure 3c) (Zahner, Harkins, and Pringle 1996). For *CDC19*, 5-FU and *RRP6* deletion have similar effects, albeit at different levels, while *BUD9* displays strong 5-FU mediated repression and inverted cell cycle regulation in the absence of functional Rrp6.

It is noteworthy that *BUD9*'s overlapping antisense lncRNAs SUT551 and XUT1242 accumulate in 5-FU treated and *rrp6*

mutant cells and that the sense/antisense transcripts form a stable dsRNA in dividing but not differentiating yeast cells and in haploid cells undergoing mitotic growth (Figure 3c,d; Figure S1). The biological role of this dsRNA, if any, is unclear but it might reflect an additional layer of *BUD9* regulation during the yeast life cycle.

These examples highlight 5-FU<sup>R</sup>'s potential applications alone and in combination with dsRNA profiling data ([http://vm-gb.curie.fr/mw2/small\\_RNA-seq](http://vm-gb.curie.fr/mw2/small_RNA-seq); <http://sensr.genouest.org>).

### 3.4 | Mitotic Repression of Meiotic Genes

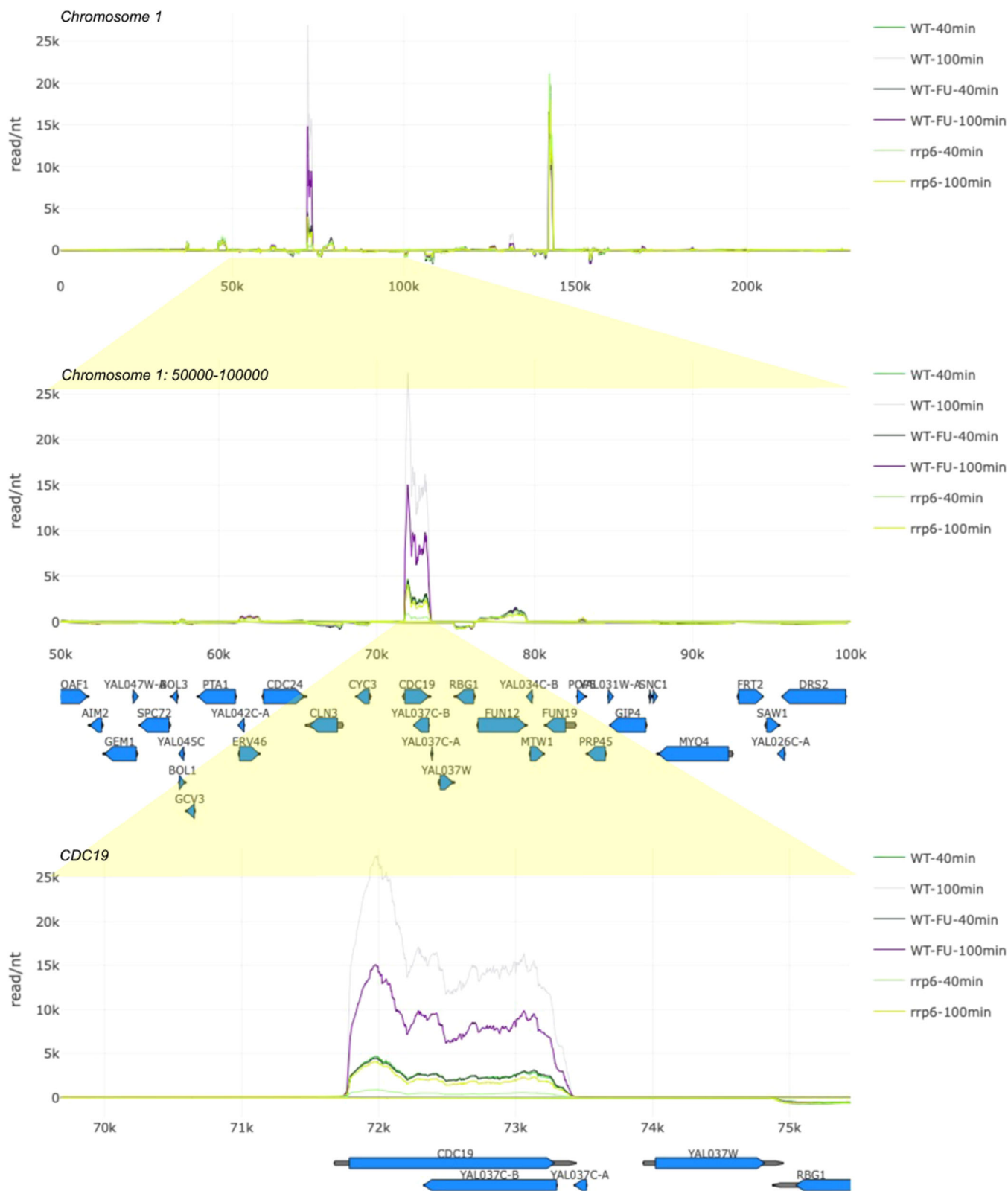
Another noteworthy aspect of 5-FU<sup>R</sup>'s data set is that diploid cells can exit mitosis and enter meiotic differentiation in response to nutritional cues, while haploid cells do not have that option. Meiosis-specific genes are therefore repressed under all conditions assayed in the data set but cells have the potential to rapidly induce these genes. The classical view is that early and middle meiotic genes are repressed in cycling cells by the tripartite histone deacetylase Rpd3/Sin3/Ume6 and Sum1/Hst1, respectively (Kadosh and Struhl 1997; J. Xie et al. 1999). However, *NDT80*, encoding an activator of middle meiotic genes (Chu and Herskowitz 1998), almost completely overlaps antisense SUT587, which appears to be transcribed from a mitotic bidirectional promoter located within *NDT80* that drives antisense SUT587 and an as yet unannotated CUT-like sense transcript (Figure 4a,b). Bidirectional transcription that initiates within the coding sequence might contribute to the repression of *NDT80* during mitotic growth of diploid cells via a variation of the classical antisense transcription interference mechanism.

Furthermore, the mRNAs of *SPO22* and *HOP1*, which are early meiotic genes important for chromosome synapsis (Hollingsworth, Goetsch, and Byers 1990; Tsubouchi, Zhao, and Roeder 2006), form dsRNAs with their antisense lncRNAs SUT183 and SUT611, respectively. These SUTs increase in the presence of 5-FU and *rrp6* mutant cells (Figure 5a,b). It is therefore conceivable that antisense interference and/or dsRNA formation contribute to the regulation of *SPO22* and *HOP1* during mitosis and meiosis in diploid cells and in cycling haploid cells (Figure S2). Such data may inspire further work on the role of antisense interference in mitotic repression of meiotic genes.

### 3.5 | A Variety of ncRNAs Respond to 5-FU and/or Deletion of *RRP6*

Earlier microarray-based RNA profiling work showed that yeast Rrp6 degrades and processes diverse ncRNAs, including transfer RNA (tRNA) precursors (Gudipati et al. 2012). In this context it is interesting that 5-FU treatment and *RRP6* deletion upregulate *SUF2* (albeit to very different levels; Figure 6a). This is a gene encoding a proline tRNA that was isolated as a suppressor of frameshift mutations induced by the DNA alkylating mutagen ICR-170 (Culbertson et al. 1977; Cummins and

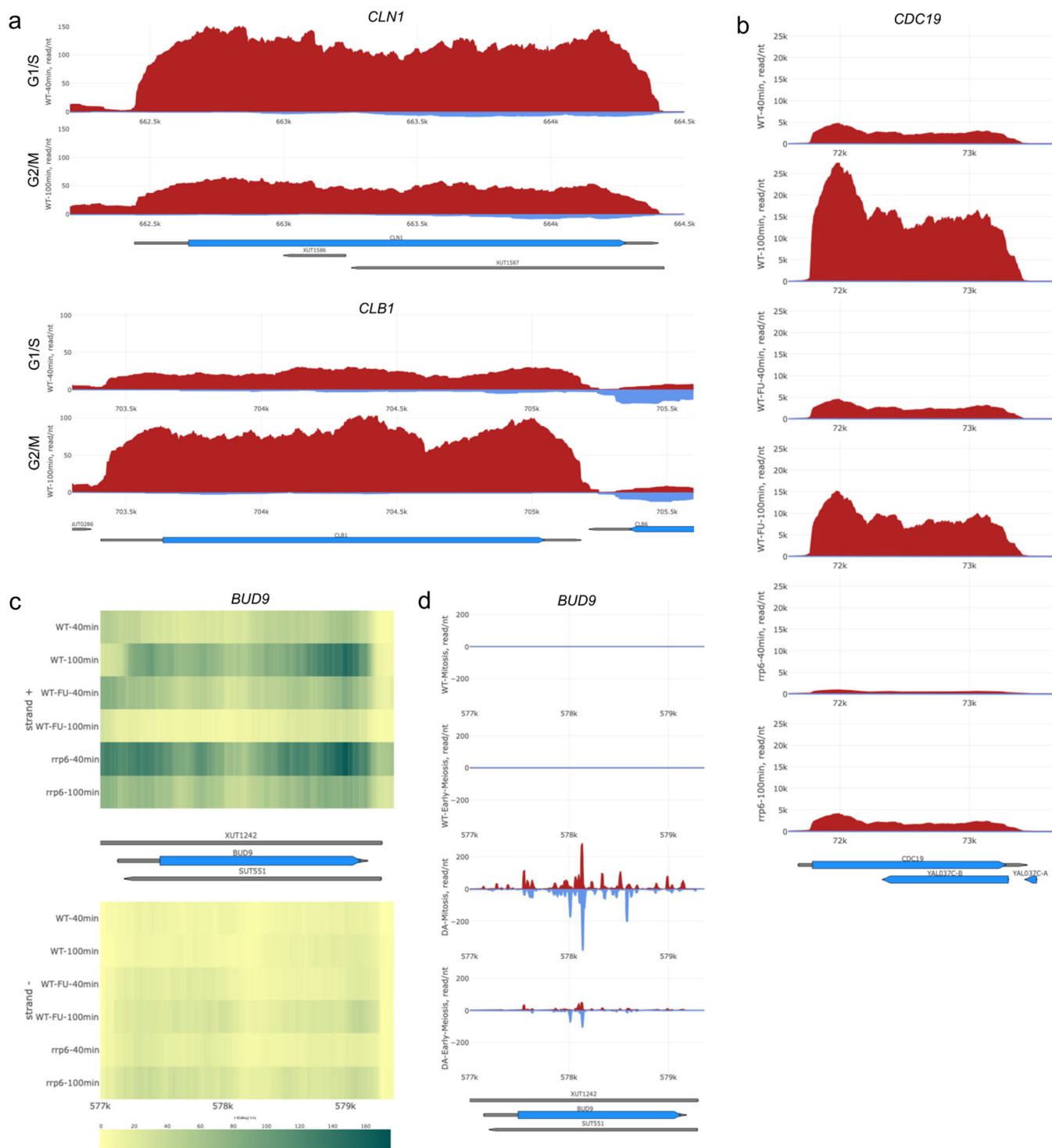




**FIGURE 2** | Chromosome-wide queries. A work-flow is shown that ranges from Chromosome 1 (top) to selected coordinates (middle) and to one target gene (bottom) as indicated. The data are displayed as color-coded line diagrams plotting genome coordinates (x-axis) against signal intensities (read/nt, y-axis). A legend shows sample annotation. Blue bars indicate open reading frames.

Culbertson 1981). It is tempting to speculate that *SUF2*'s accumulation is part of the cellular stress response to treatment with 5-FU, which causes DNA lesions, and dysfunctional RNA processing in the absence of Rrp6.

5-FU<sup>R</sup> is also suitable as a tool to compare relative levels of classical Rrp6 targets (such as CUT442) and other types of ncRNAs that are either thought to be stable or to be targeted by Xrn1 (such as SUT004 and XUT0014; Figure 6b).

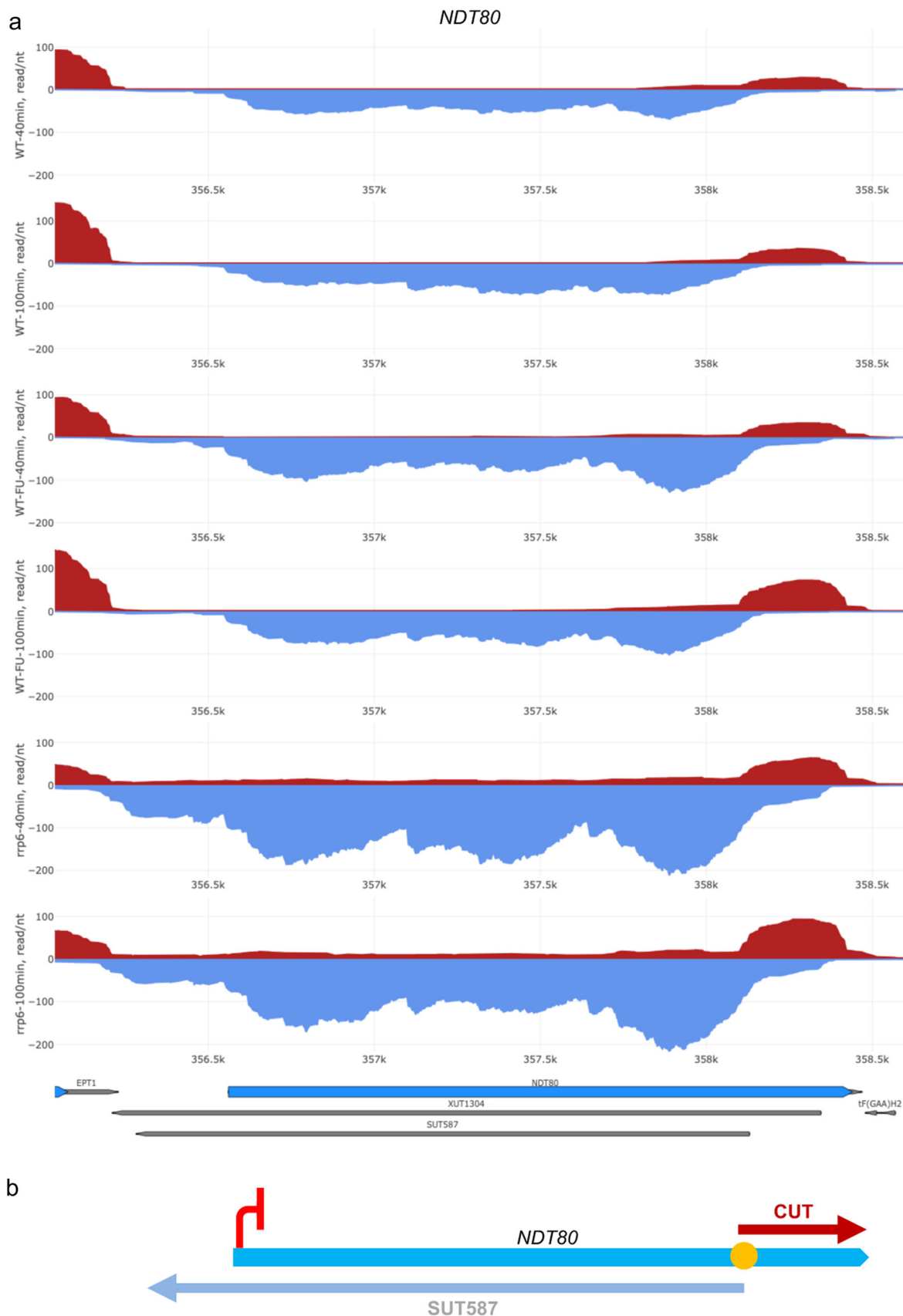


**FIGURE 3** | Mitotic cell cycle regulation. (a, b) Selected cell cycle regulated target genes are shown as color-coded filled line diagrams that plot the genome annotation data (*x*-axis) against linear expression data (*y*-axis). Samples are like in Figure 2. The mitotic cell cycle phases are indicated to the left. (c) A heatmap covers sense/antisense RNA's linear expression levels detected at the *BUD9* locus in wild type (WT), 5-FU treated (WT 5-FU) and *rrp6* samples at two time points (40 and 100 min) given to the left. Top (+) and bottom (-) strands are indicated to the left. A scale is shown at the bottom. (d) Linear dsRNA signals for *BUD9* are shown as a filled line diagram that plots genome annotation data (*x*-axis) against signal intensities (read/nt, *y*-axis) in samples from mitotic and meiotic wild-type (WT) and mitotic and meiotic Dicer/Argonaute (DA) strains as given to the left.

### 3.6 | 5-FU Triggers a Multipronged DNA Damage Response and Affects Amino Acid Biosynthesis

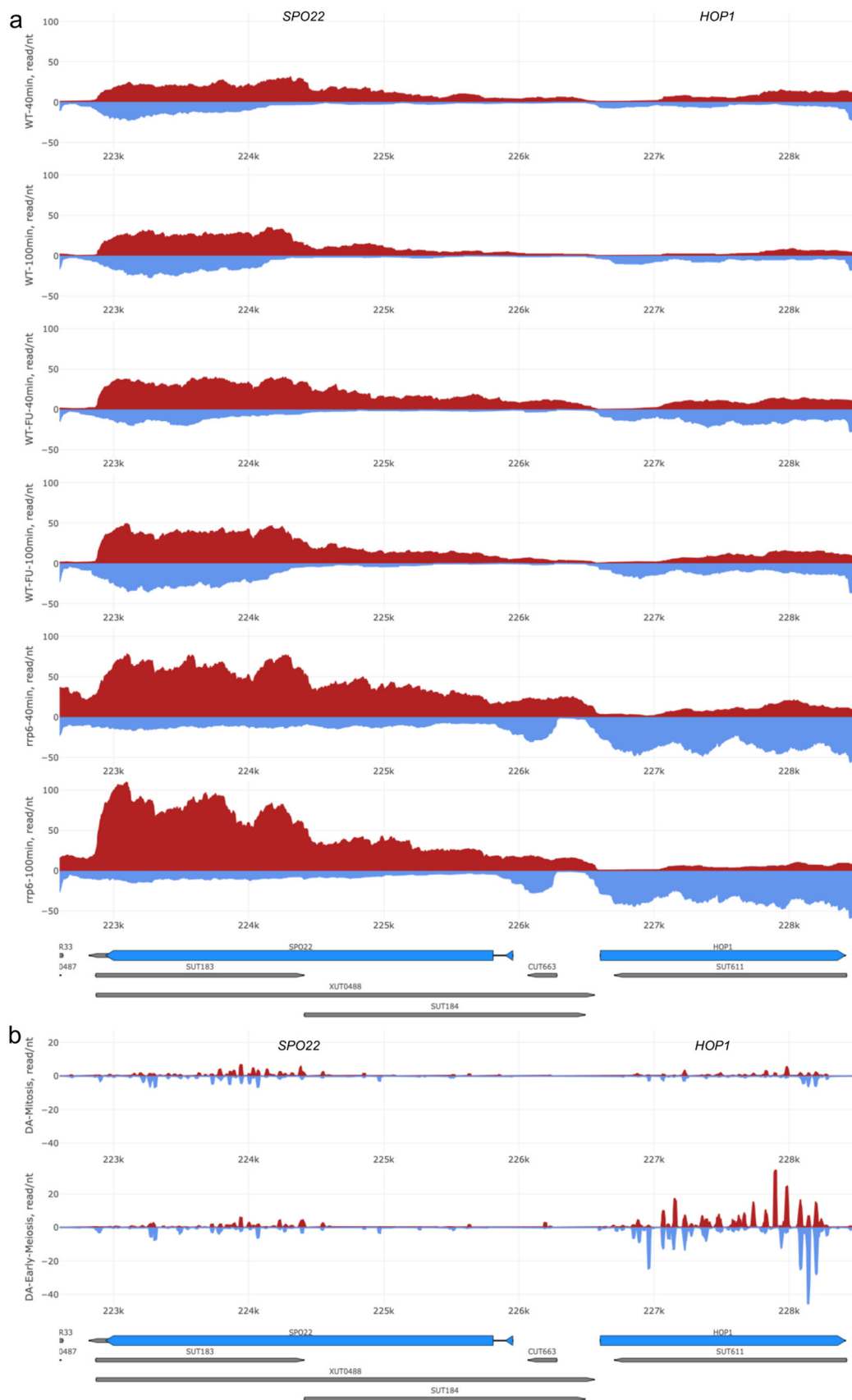
5-FU is metabolized into several nucleotide derivatives, such as fluorouridine triphosphate (FUTP), which can be incorporated

into RNA instead of uridine triphosphate (UTP) or into DNA instead of thymidine triphosphate (dTTP). The latter causes DNA lesions that trigger a broad DNA damage response; for review, see (Wilson et al. 2014). Accordingly, 5-FU treated wildtype cells, but not *rrp6* mutant cells, highly induce genes

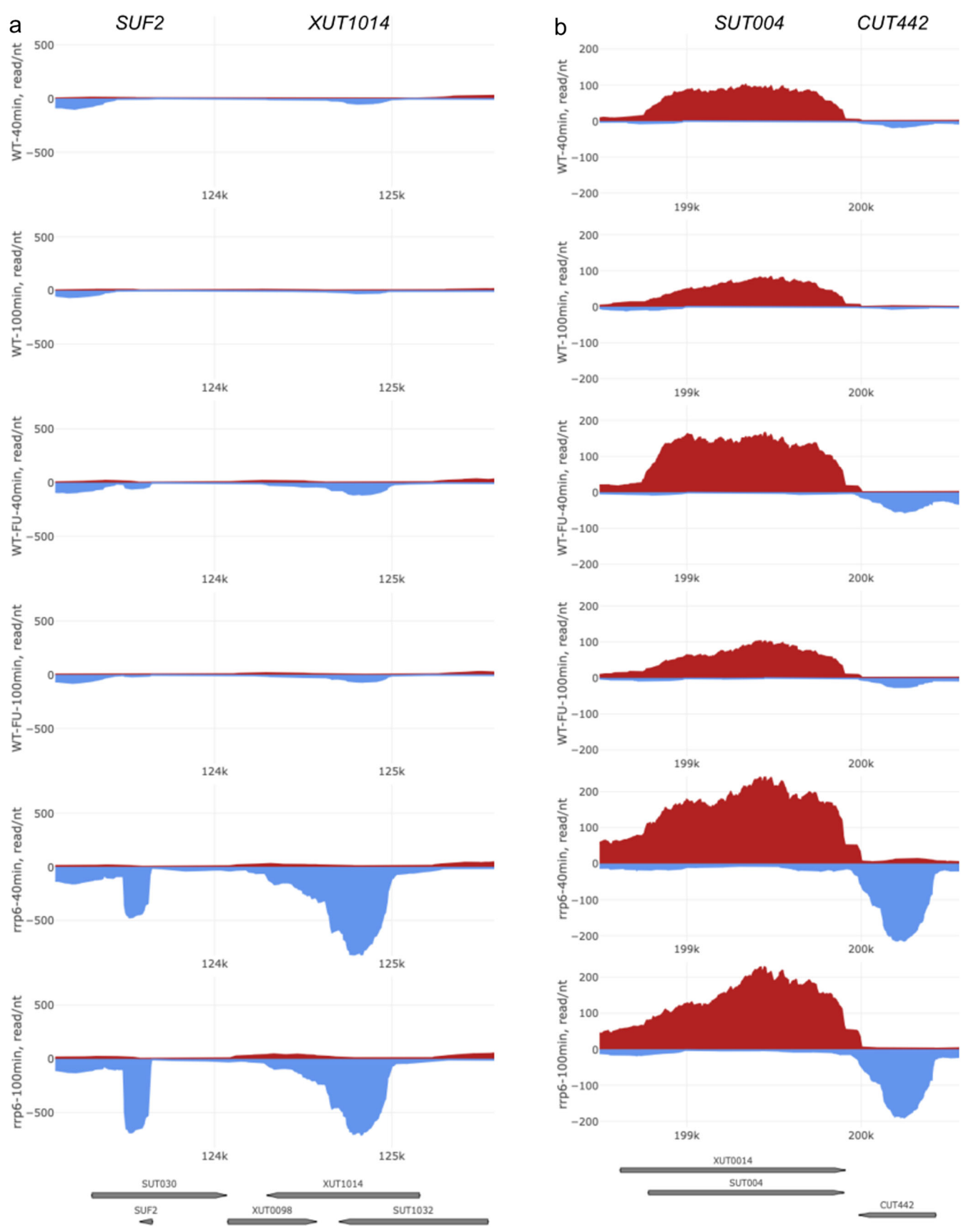


**FIGURE 4** | Expression signals for the *NDT80/SUT587* locus in mitotic cells. (a) Expression signals are shown as a filled line diagram like in Figure 3a. (b) A schematic outlines a possible mechanism contributing to *NDT80*'s mitotic repression by bidirectional transcription interference via *SUT/CUT* expression driven by a putative internal bidirectional promoter (yellow circle).

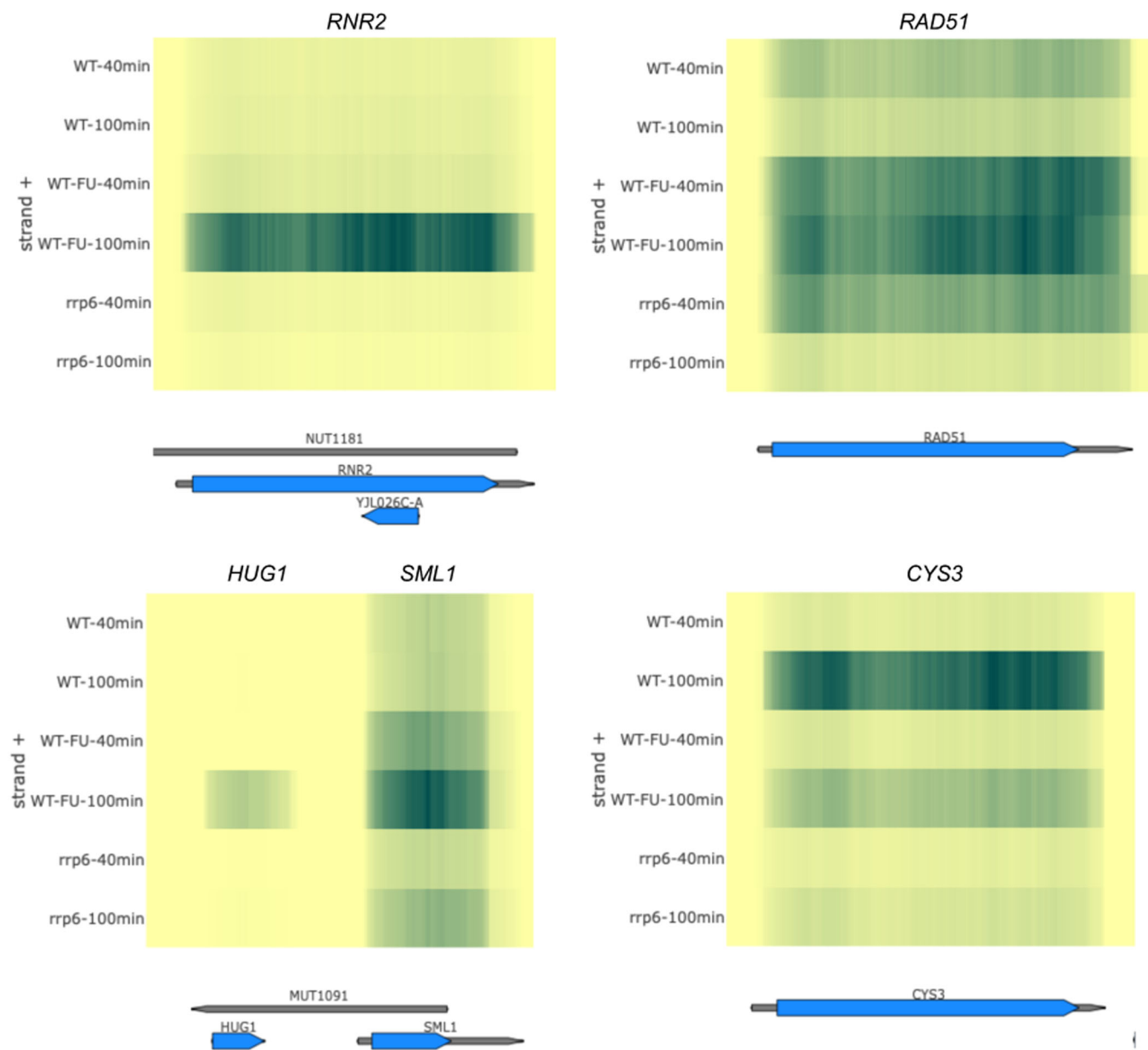




**FIGURE 5** | Mitotic sense/antisense expression patterns at meiotic loci. (a) Expression signals are shown as a filled line diagram for two target loci as indicated like in Figure 3a. (b) dsRNA signal intensities are indicated for the same loci as in Figure 3d.



**FIGURE 6** | Diverse ncRNA levels altered by 5-FU and RRP6 deletion. (a, b) Expression signals for target genes as indicated at the top are shown like in Figure 3a.



**FIGURE 7** | The 5-FU induced DNA damage response. Heatmaps are shown like in Figure 3c for selected target genes indicated at the top.

involved in *de novo* deoxyribonucleotide biosynthesis (*RNR2*), double-strand break repair via synthesis-dependent strand annealing (*RAD51*) and DNA-damage induced inhibition of ribonucleotide reductase (*HUG1*, *SML1*) (Figure 7). Conversely, 5-FU treatment and *RRP6* deletion appear to inhibit DNA replication stress induced amino acid biosynthesis (*CYS3*); for references, see <http://www.yeastgenome.org> (Engel et al. 2022).

The patterns summarized above illustrate how users who hypothesize that genes of interest may fulfill roles in the processes and pathways described, can access data that help design follow-up experiments.

#### 4 | Discussion

We describe 5-FU<sup>R</sup>, an improved tool providing access to strand-specific RNA sequencing data obtained with diploid

yeast cells cultured in the presence and absence of 5-FU in comparison to a *rrp6* mutant control strain. The experimental design was chosen because 5-FU, among other effects, inhibits the activity of yeast and fly Rrp6 (Fang, Hoskins, and Butler 2004; Silverstein, González de Valdivia, and Visa 2011) and its human ortholog EXOSC10 (Kammler, Lykke-Andersen, and Jensen 2008). While the viewer is useful on its own, it is most effective in the context of comparable expression/dsRNA data obtained with wild type and mutant cells under different growth conditions that are provided by comparable tools for strand-specific microarray and RNA-sequencing data as described above.

The present data set enables researchers to identify cell cycle regulated genes in mitotic diploid cells and to interpret the effect of an antiproliferative agent and perturbed nuclear RNA exosome function on their patterns. In addition, users can investigate DNA strand-specific expression signals at loci

harboring meiosis-specific genes in cycling cells under normal and stress conditions. This is a particularly intriguing aspect of the data set because it suggests that meiotic genes involved in processes that are conflicting with mitotic cell cycle progression, might be subject to more stringent repression mechanisms than previously thought. For example, even moderate upregulation of proteins involved in chromosome synapsis (Spo20, Hop1) or the activation of genes involved in gamete formation (Ndt80) might interfere with normal mitotic cell division. This is an interesting biological problem related to the well-studied derepression of testicular genes in somatic cancers: a subgroup of these so-called Cancer/Testis genes may provide meiotic functions that confer malignant features to malignant somatic cells. Relevant examples are genes involved in recombination (*MEIOB*, *SPO11*) and chromosome cohesion (*HORMAD1*, *SYCP2*) (Wang et al. 2024); for review, see (Feichtinger and McFarlane 2019; Gibbs and Whitehurst 2018).

Users can also combine information on sense/antisense gene expression and dsRNA formation in diploid and haploid cells to inspire work on regulatory mechanisms that control RNA synthesis, stability and mRNA translation (Drinnenberg et al. 2009; Sinturel et al. 2015; Wery et al. 2016).

In addition, researchers can identify their favorite target genes as 5-FU responders, a pattern that marks them out as potential candidates involved in DNA repair and the regulation of cell growth (Hoskins and Scott Butler 2007; Lum et al. 2004; Seiple, 2006). Finally, 5-FU<sup>R</sup> helps interpret the broad effect of impaired nuclear RNA exosome activity on global RNA degradation and processing and perturbed transcription termination (Gudipati et al. 2012; Neil et al. 2009; Xu et al. 2009).

Antiproliferative drugs that affect multiple pathways, such as 5-FU, are difficult to study as far as their mechanisms of action are concerned. Data obtained with the highly experimentally accessible budding yeast model likely facilitate efforts to gain mechanistic insights into drug cytotoxicity in eukaryotic cells. 5-FU<sup>R</sup> enables users to develop testable models about cell cycle specific gene function, regulation of gene expression, the transcriptional landscape in cells lacking a functional nuclear RNA exosome and the 5-FU drug response wild type cells trigger to survive. The design approach allows for including additional drug-related expression data sets in the future. Such work may contribute to a better understanding of how antiproliferative drugs exert their function by influencing a wide variety of protein-coding and noncoding RNAs in a simple eukaryotic cell.

## Acknowledgments

The production of experimental data provided via 5-FU<sup>R</sup> was co-funded by a grant of La Ligue Contre le Cancer (CD35 and CD29) to Michael Primig, and ANR (“DNA-life”—ANR-15-CE12-0007) and ERC (“DARK”—consolidator grant) grants to Antonin Morillon. Inserm, the University of Rennes, and EHESP provided additional support. The 5-FU<sup>R</sup> data are hosted by the GenOuest platform of Biosit at the University of Rennes.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Raw data are available via GSE77511 at the NCBI's GEO repository.

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### Supporting Information

Additional supporting information can be found online in the Supporting Information section.