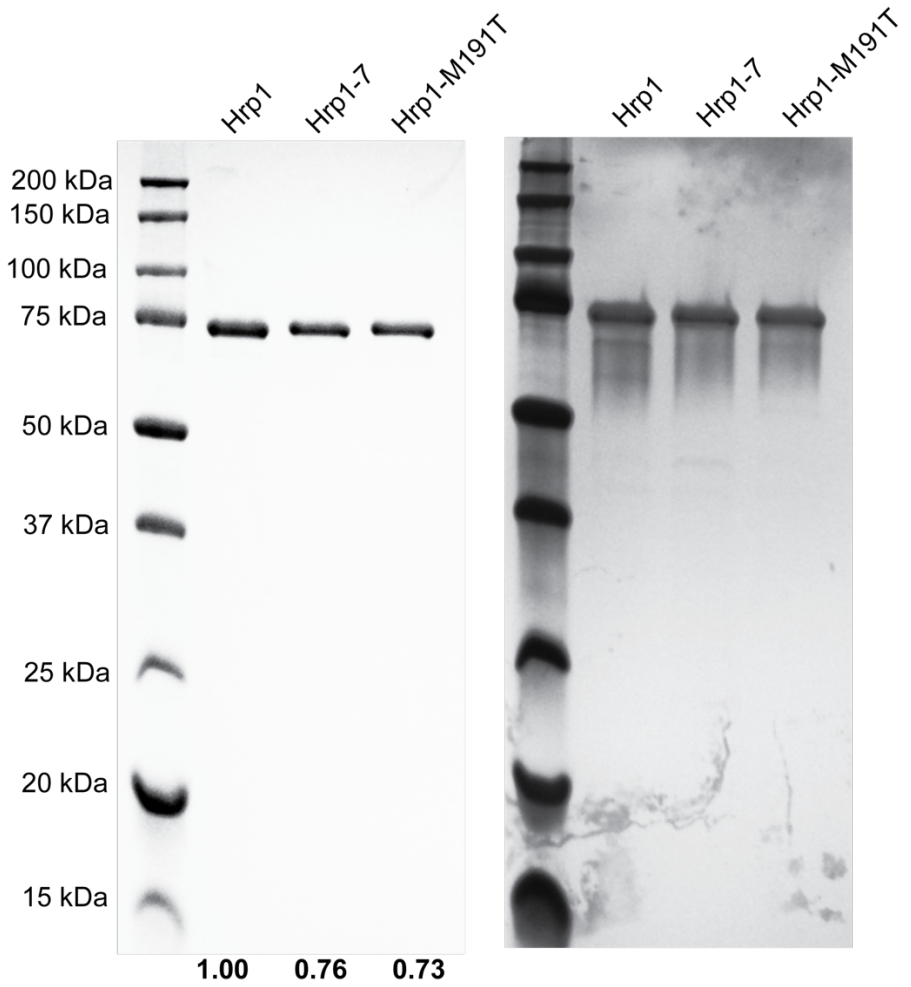
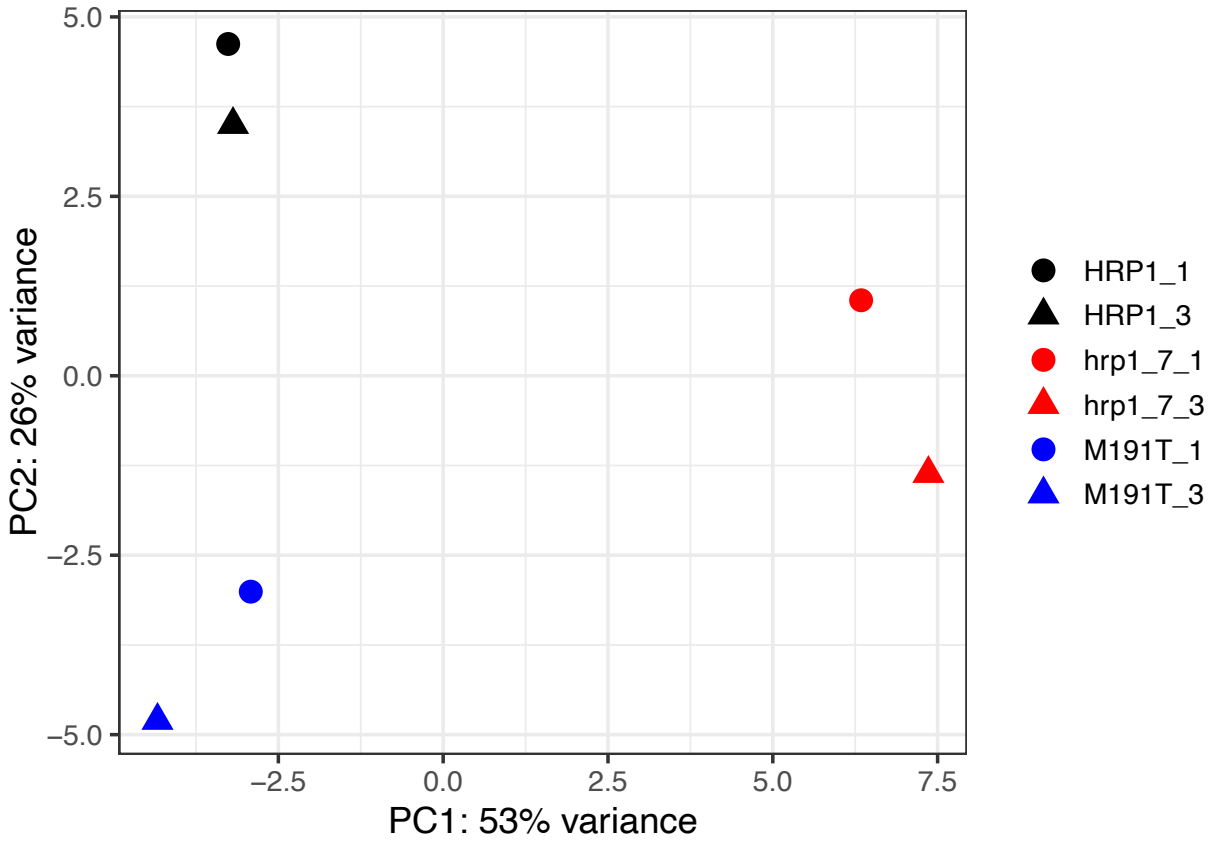
Supplemental Table S1. Fold change in *SNR* gene readthrough (RT) reads in *hrp1-7* and -M191T mutants compared to wild-type. Biological duplicates are indicated "1" and "3" and are averaged. Genes are ordered by strength of readthrough in the *hrp1-7* strain. Only *SNR* genes with ≥10 total RT reads in *HRP1* or *hrp1-7* strains are included in the table.

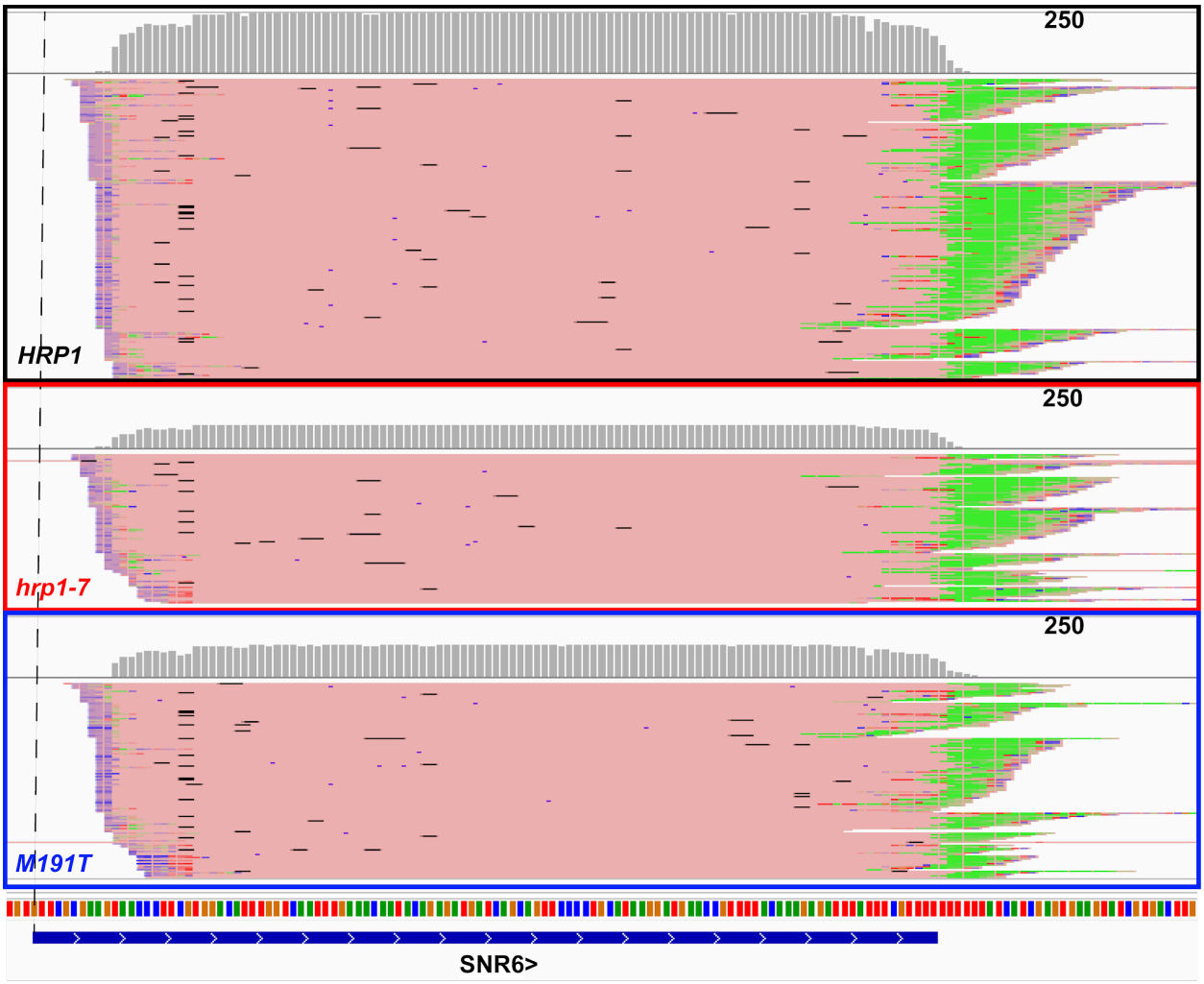
snoRNA	HRP1_1_RT	HRP1_3_RT	HRP1_RT_avg	hrp1-7_1_RT	hrp1-7_3_RT	hrp1-7_RT_avg	hrp1-7/HRP1	M191T_1_RT	M191T_3_RT	M191T_avg	M191T/HRP1
SNR82	10	9	9.5	100	101	100.5	10.6	19	16	17.5	1.8
SNR68	4	6	5	41	34	37.5	7.5	14	14	14	2.8
SNR42	1	2	1.5	8	5	6.5	4.3	1	4	2.5	1.6
SNR33	19	21	20	98	60	79	4	13	19	16	0.8
SNR80	4	2	3	13	7	10	3.3	10	15	12.5	4.2
SNR161	5	2	3.5	14	5	9.5	2.7	2	1	1.5	0.4
SNR5	6	4	5	12	7	9.5	1.9	4	3	3.5	0.7
SNR32	4	4	4	7	7	7	1.8	7	7	7	1.8
SNR71	19	18	18.5	40	22	31	1.7	27	26	26.5	1.4
SNR47	10	16	13	28	13	20.5	1.6	19	8	13.5	1
SNR64	3	6	4.5	7	5	6	1.3	2	7	4.5	1
SNR69	9	18	13.5	12	18	15	1.1	23	28	25.5	1.9
SNR17B	10	7	8.5	8	10	9	1.1	16	12	14	1.6
SNR46	21	15	18	17	20	18.5	1	30	27	28.5	1.6
SNR62	3	7	5	4	6	5	1	6	6	6	1.2
SNR87	22	29	25.5	26	20	23	0.9	45	32	38.5	1.5
SNR39B	17	19	18	16	13	14.5	0.8	15	33	24	1.3
SNR34	4	9	6.5	6	3	4.5	0.7	7	5	6	0.9
SNR60	24	30	27	21	13	17	0.6	20	27	23.5	0.9
SNR66	36	25	30.5	18	10	14	0.5	16	26	21	0.7
SNR63	28	20	24	14	6	10	0.4	25	39	32	1.3
SNR7-L	12	10	11	3	6	4.5	0.4	11	20	15.5	1.4
SNR7-S	12	10	11	3	6	4.5	0.4	11	20	15.5	1.4
SNR10	14	41	27.5	11	10	10.5	0.4	20	16	18	0.7
SNR3	7	8	7.5	3	2	2.5	0.3	0	4	2	0.3
SNR30	41	35	38	3	5	4	0.1	8	19	13.5	0.4
SNR56	13	24	18.5	1	2	1.5	0.1	5	6	5.5	0.3



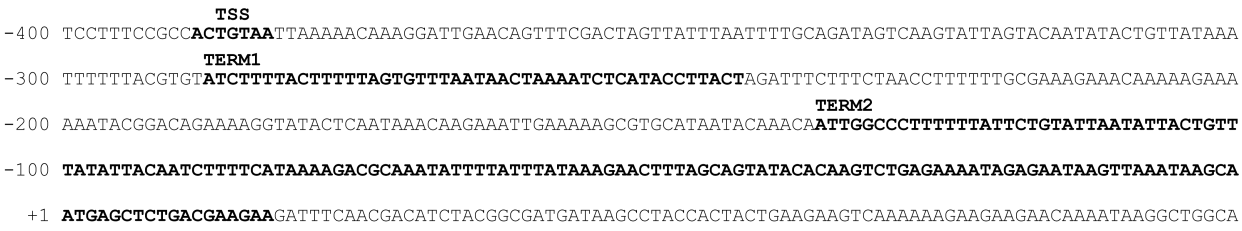
Supplemental Figure S1. SDS-PAGE analysis of recombinant Hrp1 proteins used for the RNA-binding experiments in Figure 1. Each lane was loaded with 12.5 pmol protein as determined by the calculated extinction coefficient at 280 nm. The gel was stained with Coomassie blue (left) then silver (right). Quantification of band intensities from the Coomassie-stained gel was performed in ImageJ, normalized to the wild-type Hrp1 signal, and reported below each lane. These values were used to retrospectively adjust the protein concentration for the K_D analysis.



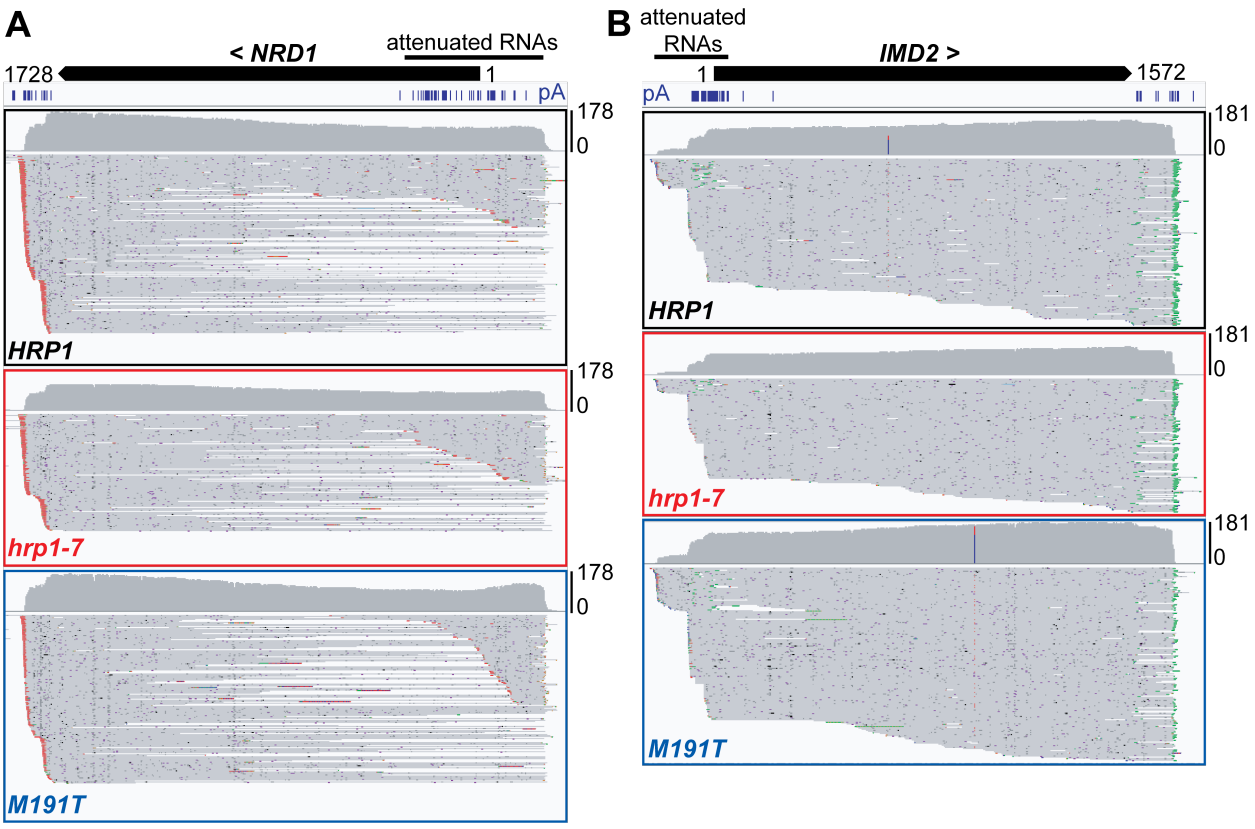
Supplemental Figure S2. Principle component analysis of direct RNA-seq reads over genes shows that biological replicates are much more similar than strains with different *HRP1* genotypes. Variance in the number of reads over 5,489 protein-coding genes between samples was determined using DESeq2.



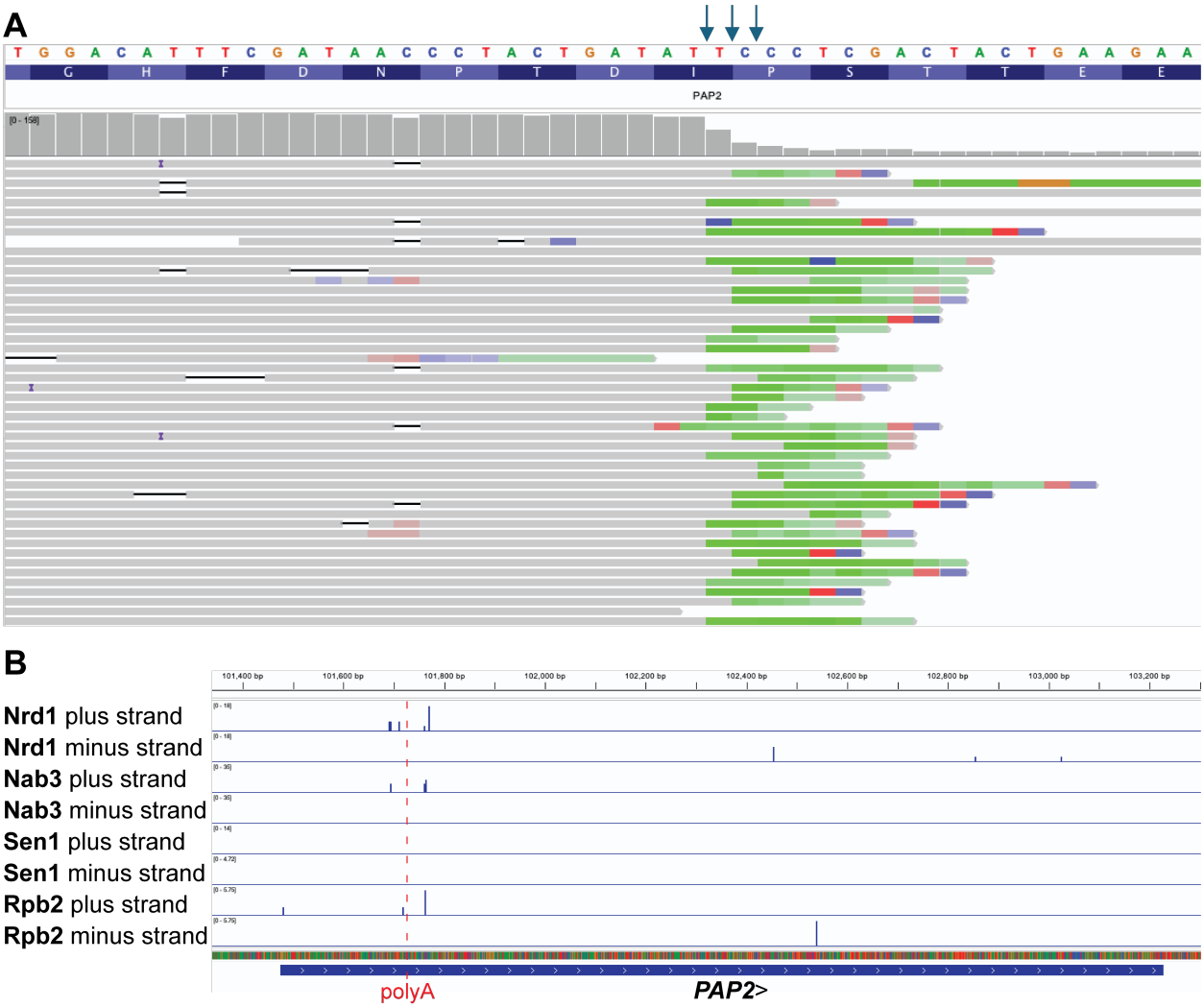
Supplemental Figure S3. IGV browser view showing dRNA-seq reads aligned to the U6 snRNA gene, *SNR6*, in isogenic strains with the indicated *HRP1* genotype. Not all individual reads are shown. The blue bar at the bottom shows the extent of mature U6 snRNA, the colored marks above it represent the nucleotide sequence, and the dotted line marks the first nucleotide of mature U6. The polyA tail is colored green. The gray histogram at the top of each panel shows the number of reads for each nucleotide. The scale is 0-250 reads for all three panels. The reason for the decreased abundance of the polyadenylated U6 snRNA in the mutant strains is not known.



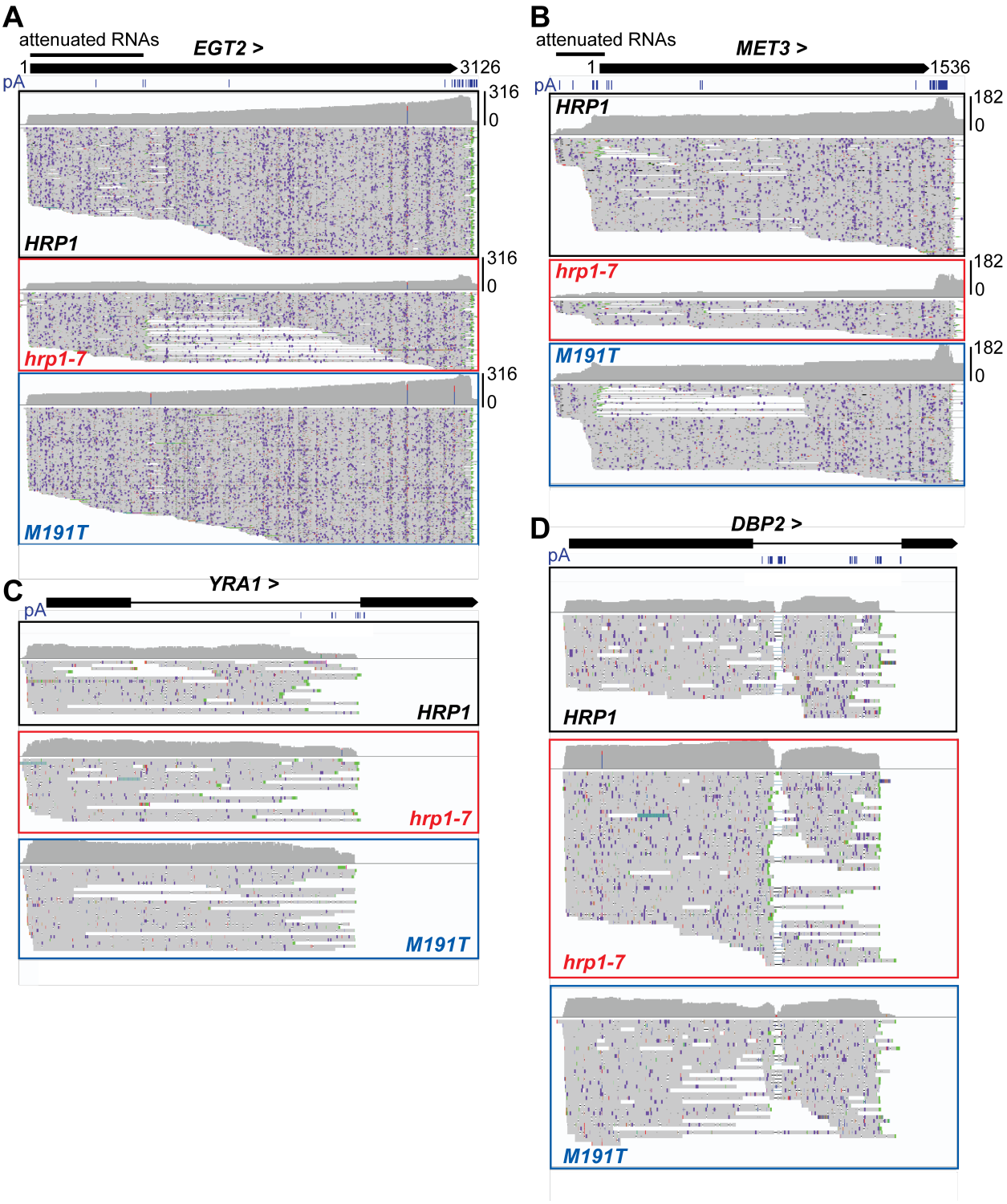
Supplemental Figure S4. DNA sequence of the *HRP1* 5'-UTR. The transcription start site region (TSS) and two main attenuation regions (Term 1 and Term 2) are in bold. Numbering is relative to the ATG start codon (+1).



Supplemental Figure S5. *NRD1* and *IMD2* attenuated transcripts are not as abundant as their mRNAs. The heavy lines demarcate the ORFs and the lighter lines above demarcate the attenuated transcripts, which are interleaved with the mRNAs. Note that *NRD1* is transcribed leftward and *IMD2* is transcribed rightward. Leftward polyA tails are colored red and rightward are colored green. The *NRD1* attenuated transcripts use the same transcription start site, while the *IMD2* attenuated transcripts use an upstream start site (Kuehner and Brow 2008).



Supplemental Figure S6. The attenuated transcript of *PAP2* has a discrete 3'end that colocalizes with Nrd1 and Nab3 crosslinks. A) 3' end positions of truncated *PAP2/TRF4* reads. The three most frequent start sites of the polyA tail (in green) are indicated with arrows. Full-length mRNAs are gray lines extending out of view to the right. **B)** In vivo PAR-CLIP crosslinking of Nrd1, Nab3, and the Rpb2 subunit of RNAP II (but not Sen1) to Pap2 mRNA (Creamer et al. 2011) in the vicinity of the *PAP2* intragenic polyA site, indicated by the red dotted line, visualized with IGV. Note that the PAR-CLIP reads are in the plus strand, corresponding to the Pap2/Trf4 mRNA.



Supplemental Figure S7. Reads from mRNAs that are decreased in the hrp1-7 strain. **A**) Attenuated reads of *EGT2* are apparent in the *hrp1-7* strain. **B**) *MET3* reads from an upstream start site are preferentially attenuated. **C,D**) Reads resulting from intronic polyA site use in *YRA1* and *DBP2* are fragmented, suggestive of decay intermediates.