

Expanded View Figures

Figure EV1. Annotation of transcripts.

- A Segmentation workflow. The Watson and Crick strands are in dark blue and green, respectively. The top 8 tracks show antisense-corrected TT-seq data tracks (log₂ scale) that were used as input for GenoSTAN. The other tracks indicate the stepwise annotation of transcripts. From the GENCODE annotation, only full transcripts with transcript_support_level 1 are depicted.
- B Jaccard index (compared to GENCODE annotation) for different choices of thresholds (x-axis: reads per kilobase (RPK)). The red line indicates the selected RPK value where the Jaccard index reaches the maximal value.
- C Number of transcripts per transcript class.
- D Distribution of transcript lengths per transcript class. Box limits are the first and third quartiles, the band inside the box is the median. The ends of the whiskers extend the box by 1.5 times the interquartile range.

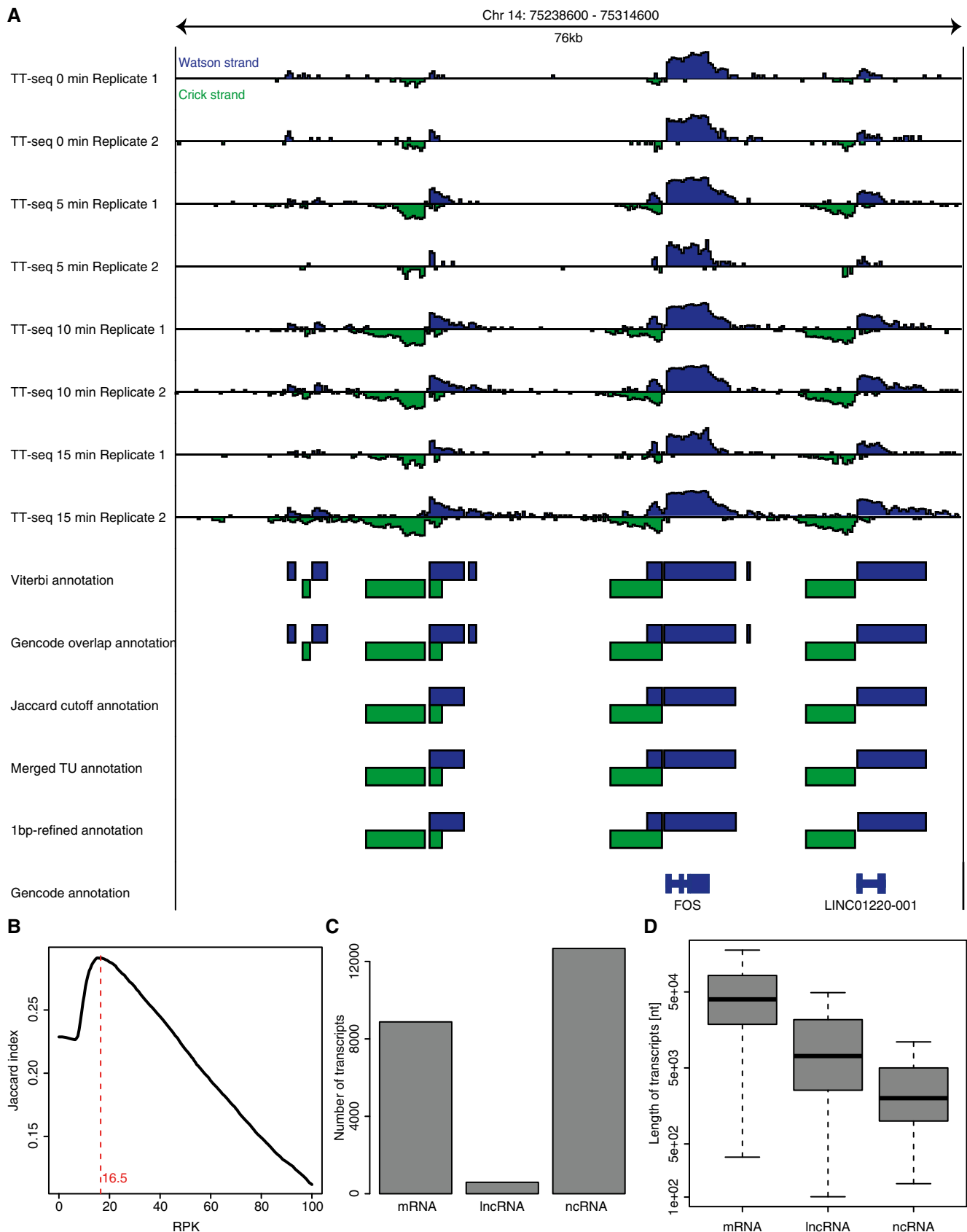
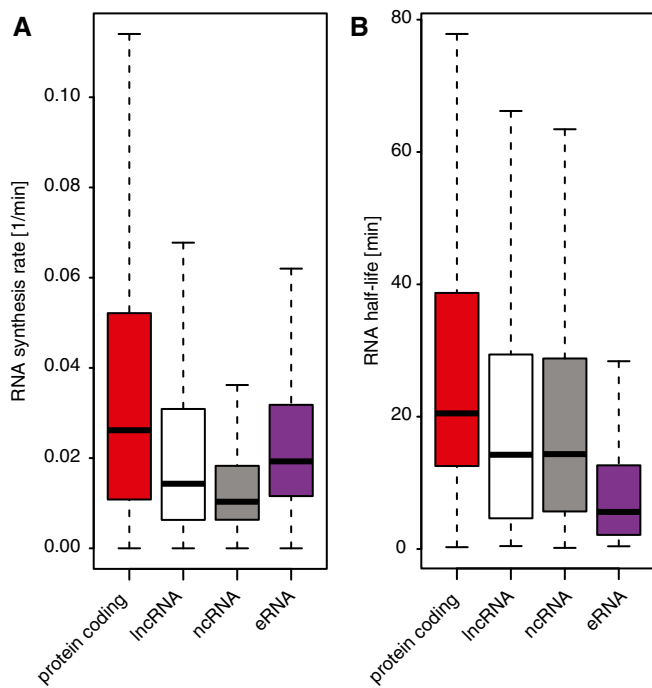


Figure EV1.

**Figure EV2. Half-life and synthesis rate distribution of transcript classes.**

A Distribution of synthesis rates for different transcript classes. See Materials and Methods for details.

B Distribution of half-lives for different transcript classes. See Materials and Methods for details.

Data information: Box limits are the first and third quartiles, the band inside the box is the median. The ends of the whiskers extend the box by 1.5 times the interquartile range.

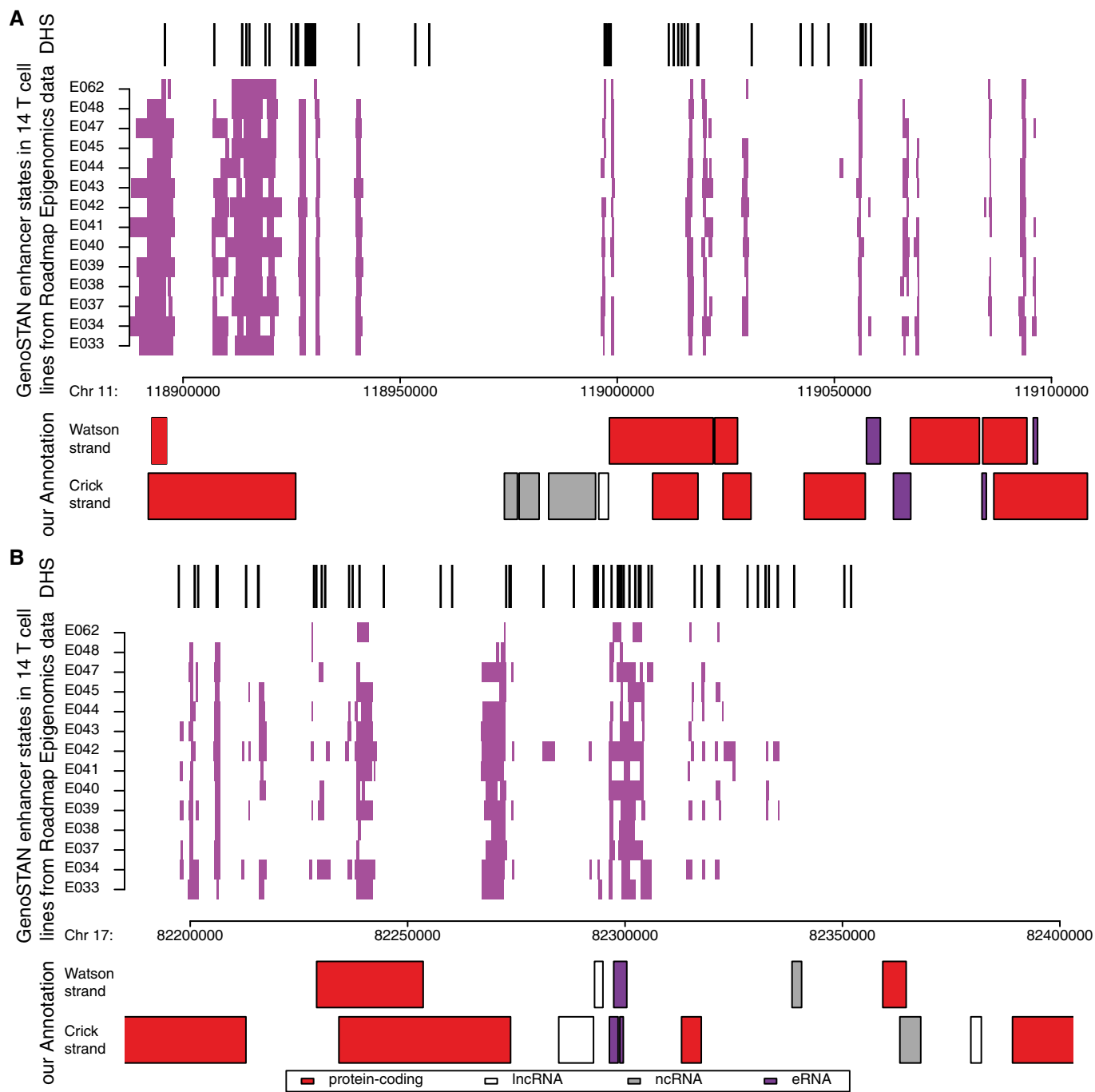


Figure EV3. Example of eRNA identification using GenoSTAN.

A Shown are the DNase hypersensitivity signal (DHS) peaks from ENCODE (top), the GenoSTAN enhancer states (violet colored) obtained from 14 Roadmap Epigenomics T-cell lines (middle), and the obtained transcript annotation (bottom) for a region on chromosome 11.
 B As in (A) for a region on chromosome 17.

Figure EV4. Details on transcribed enhancer–promoter pairs.

- A Number of transcribed enhancers and transcribed promoters per ChIA-PET-defined insulated neighborhood. Count values were jittered for visualization purposes.
- B Location and orientation of transcribed enhancers with respect to their paired target gene TSS. Negative values indicate the transcribed enhancer location upstream of the promoter TSS, positive values downstream of the promoter TSS. Upper histogram: Distance distribution for transcribed enhancers on the same ("sense") strand as their target promoter. Lower histogram: Distance distribution for transcribed enhancers on the opposite, antisense strand.
- C Distribution of the Pearson correlation between observed TT-seq signal at transcribed enhancers and promoters for the enhancer–promoter pairs derived here (where both eRNA and mRNA change significantly between 0 min and 15 min time points; red line) and for 1,000 randomly shuffled enhancer–promoter pairs (gray lines). The colored profile indicates the quantiles (5–95% of the data) with the black median line and the gray 25% and 75% quantile lines. Observed correlations are enriched for positive correlations (right peak) and depleted for negative correlations (left peak).
- D Distribution of insulated neighborhood size by cohesin-ChIA-PET and CTCF-ChIP-seq. The median size of an insulated neighborhood was 255 kb.
- E Distance of transcribed enhancer–promoter pairs versus the size of the corresponding insulated neighborhood. The lines indicate the size of the insulated neighborhood (by which the distance is bound by definition), and a third of the size of the insulated neighborhood (which is the expected distance between two randomly drawn positions in a neighborhood). The *P*-value was derived by a one-sided Mann–Whitney *U*-test. For visualization purposes, 127 points with insulated neighborhood size > 2,000 kb are not shown.

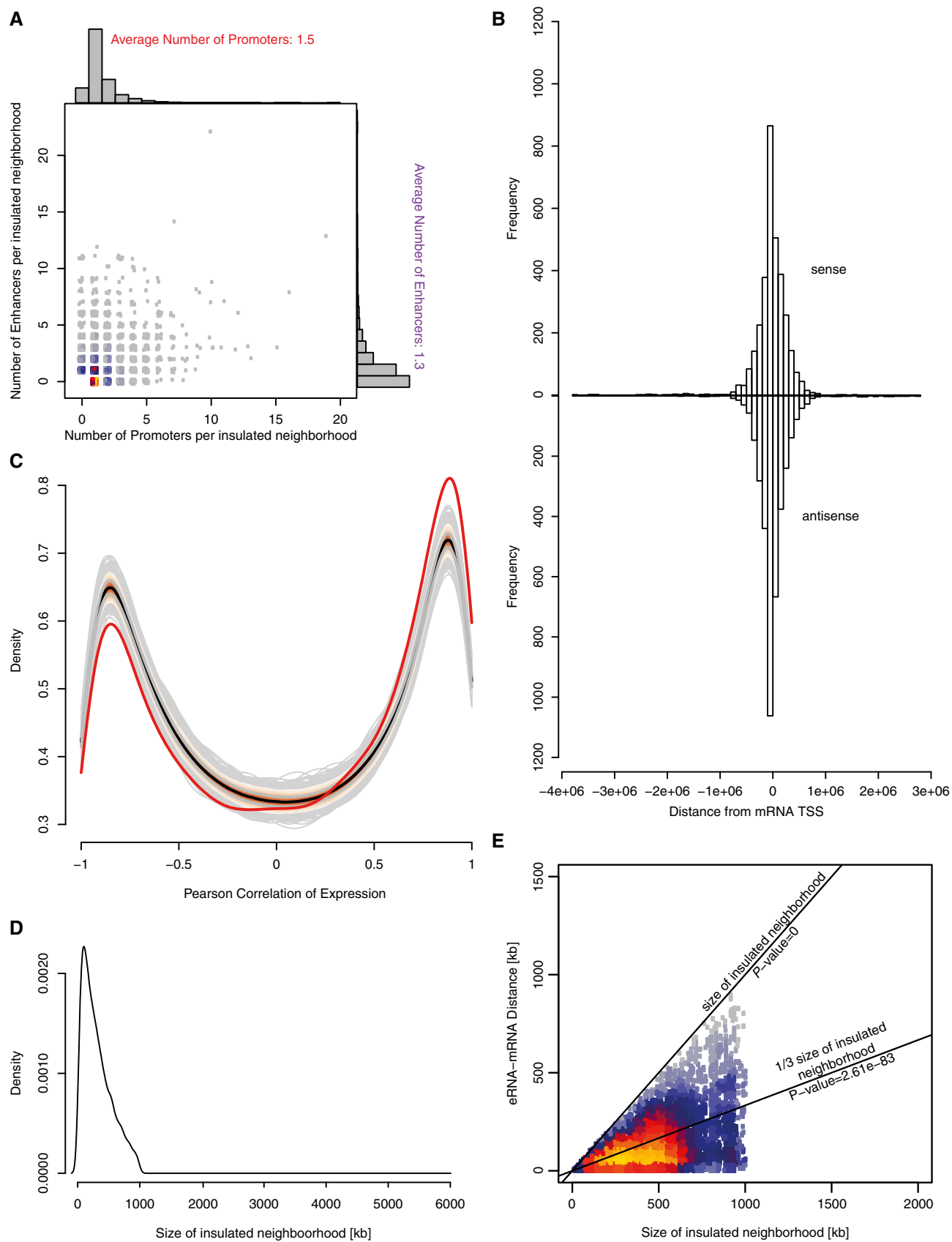


Figure EV4.