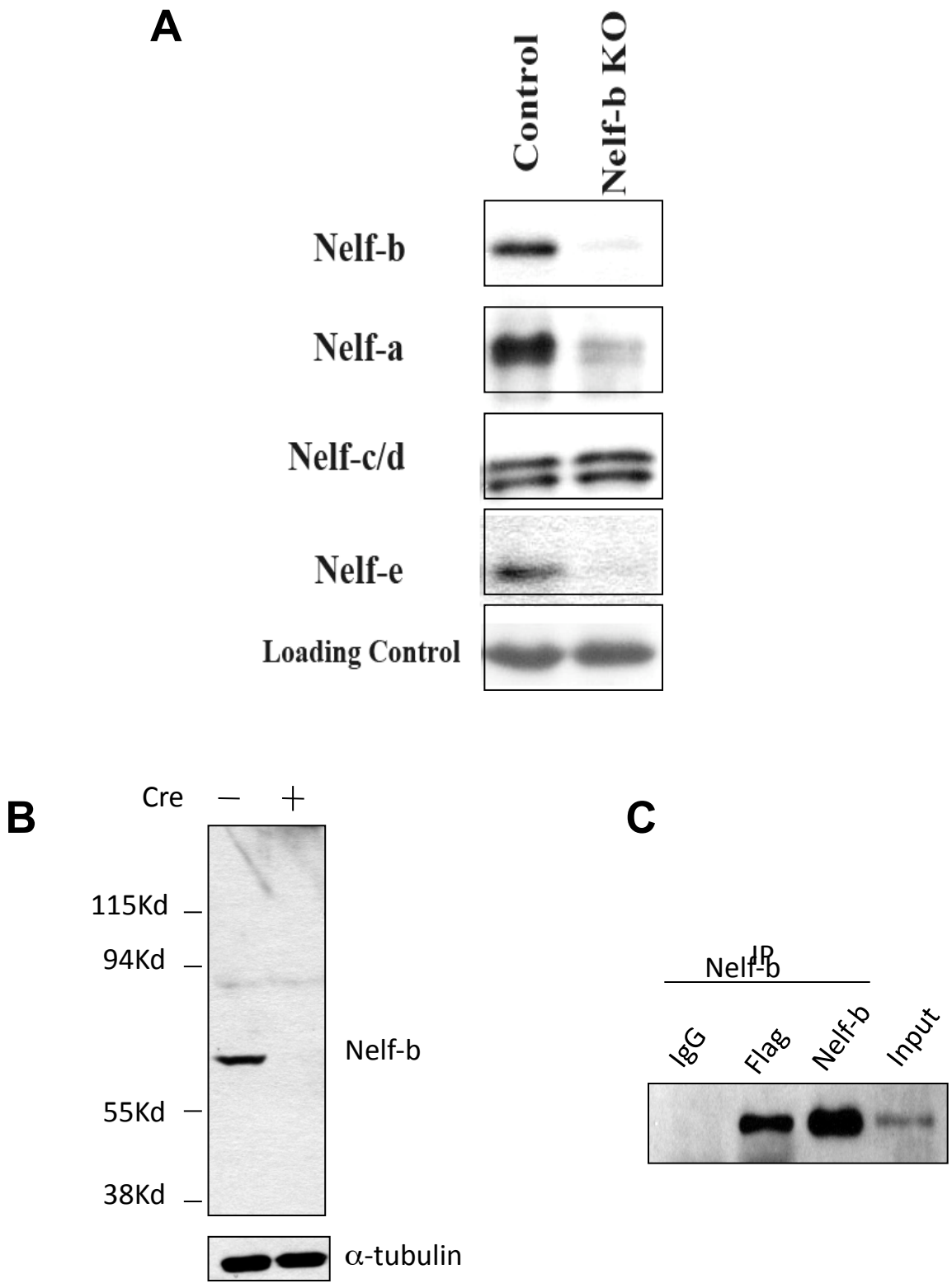

Figure S1. Validation of Nelf-b antibody for ChIP-Seq. (A). Western blots illustrate the effect of Nelf-b KO on the protein levels of all four Nelf subunits. An unknown cross-reacting protein band was included as an indicator of equal loading. (B) Western blot of Nelf-b in control and Nelf-b KO MEFs demonstrates the antibody specificity. a-Tubulin was included as loading control. (C) Comparison of immunoprecipitation efficiency of anti-Flag and Nelf-b antibodies. Whole cell extract was prepared from MEFs stably expressing Flag tagged Nelf-b. Flag-Nelf-b was then immunoprecipitated with IgG control, anti-Flag, or anti-Nelf-b antibody. Pull-downs and Input were probed with an anti-Flag antibody.

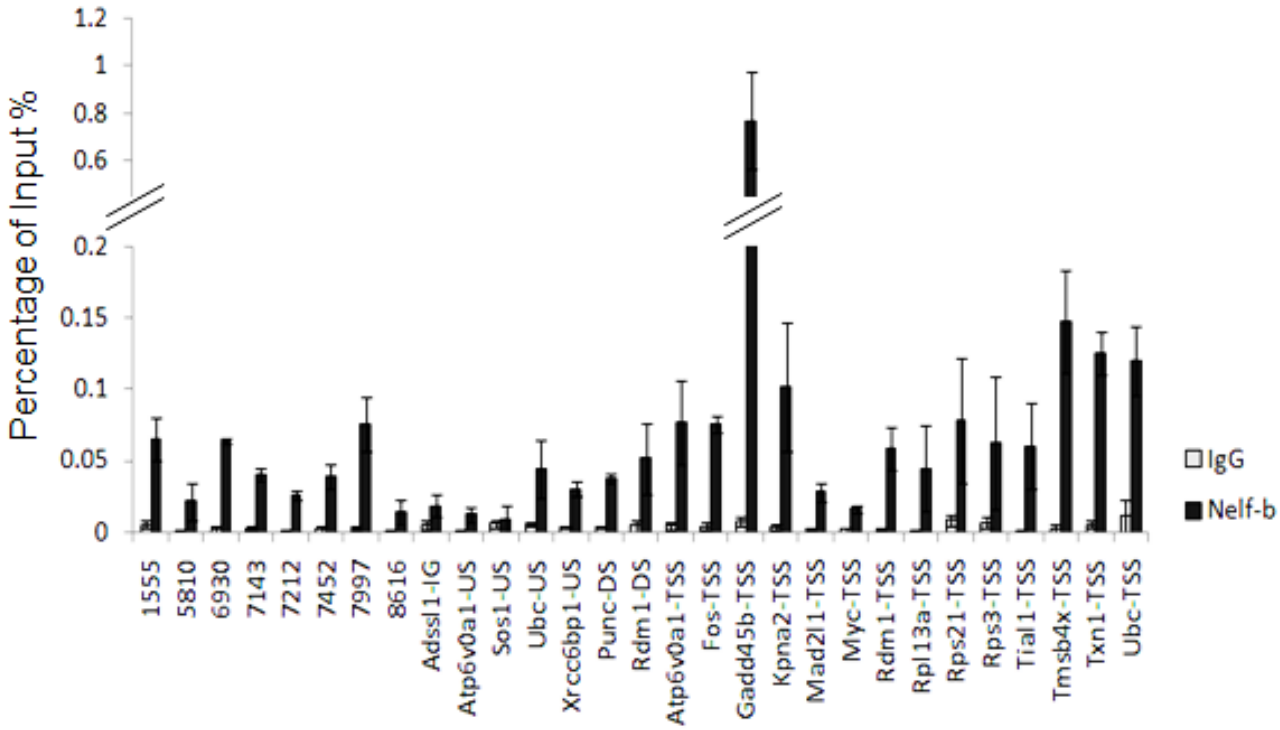

Figure S2. Validation of the ChIP-Seq results by site-specific ChIP. Normal IgG was used as a negative control. At each locus tested, the level in the control was set as 1 and that in the KO cells was expressed as fold of change over the control. Results shown were average of three experiments. Error bar: s.e.m.

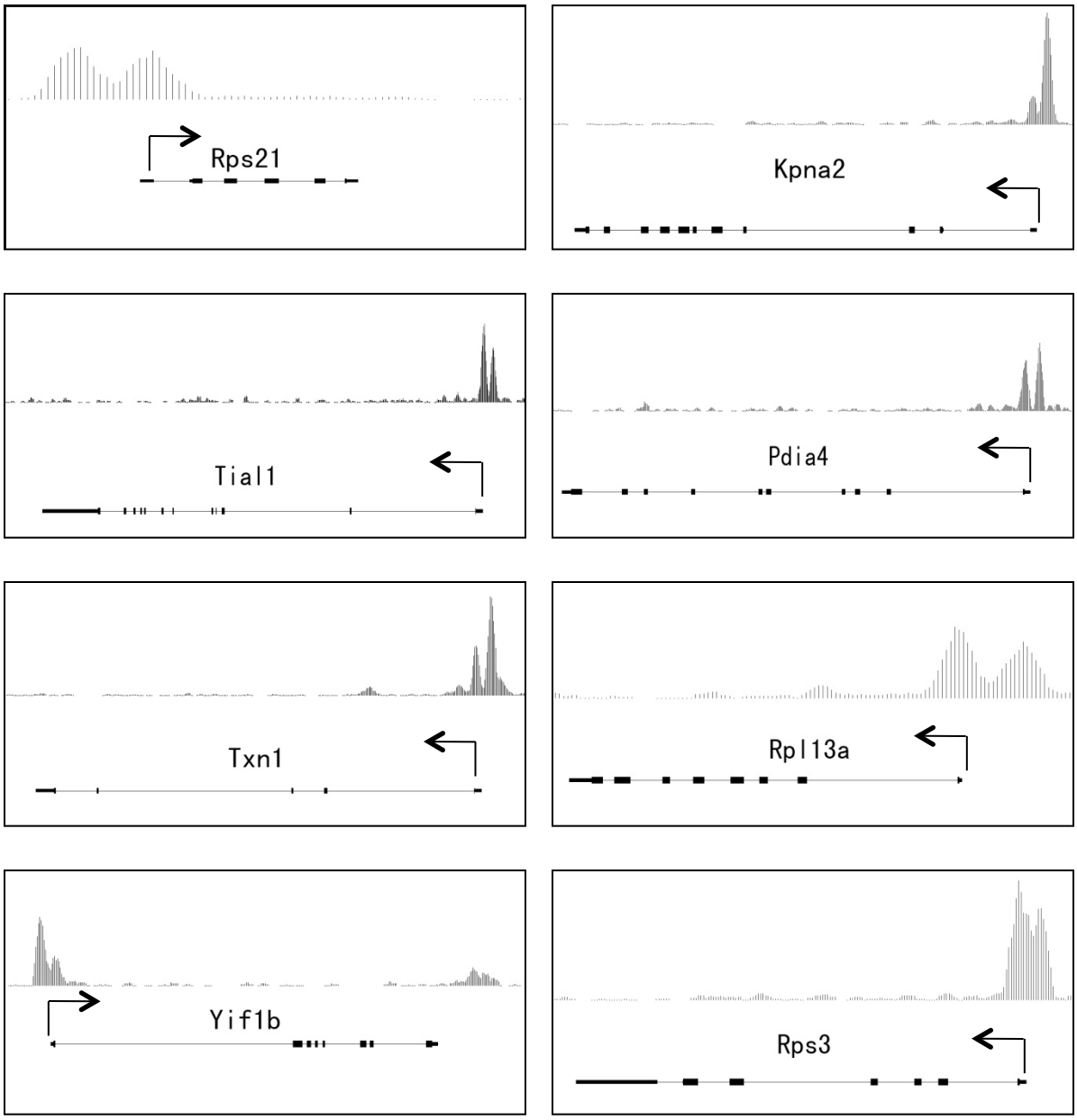

Figure S3. IGB view of twin-peak Nelf-b binding regions. Arrows indicate the transcription direction.

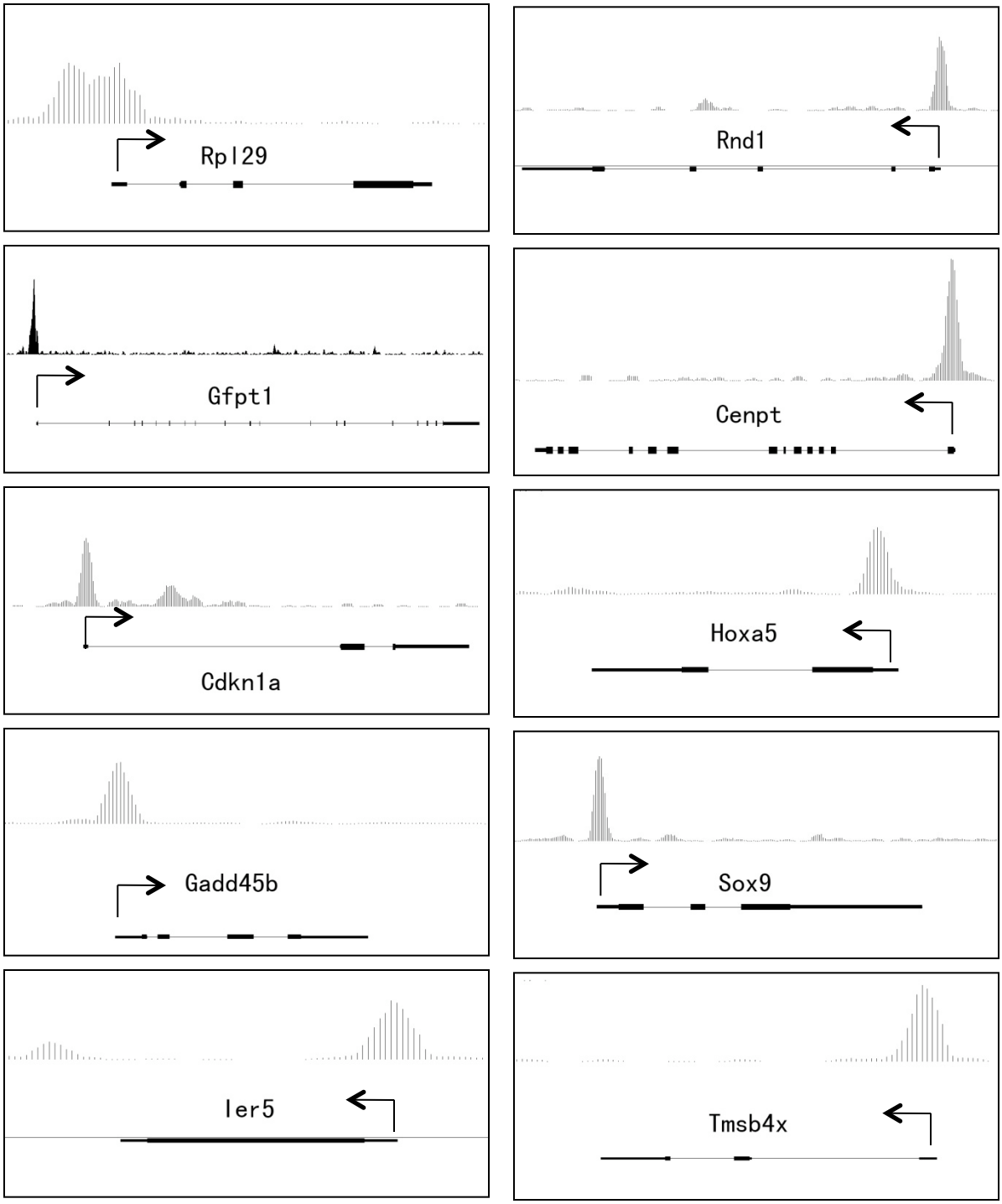

Figure S4. IGB view of single-peak Nelf-b binding regions. Arrows indicate transcription direction. Note that, despite the complex binding pattern of Nelf-b at the Rpl29 promoter, our algorithm classified it as a single-peak Nelf-b binding region.

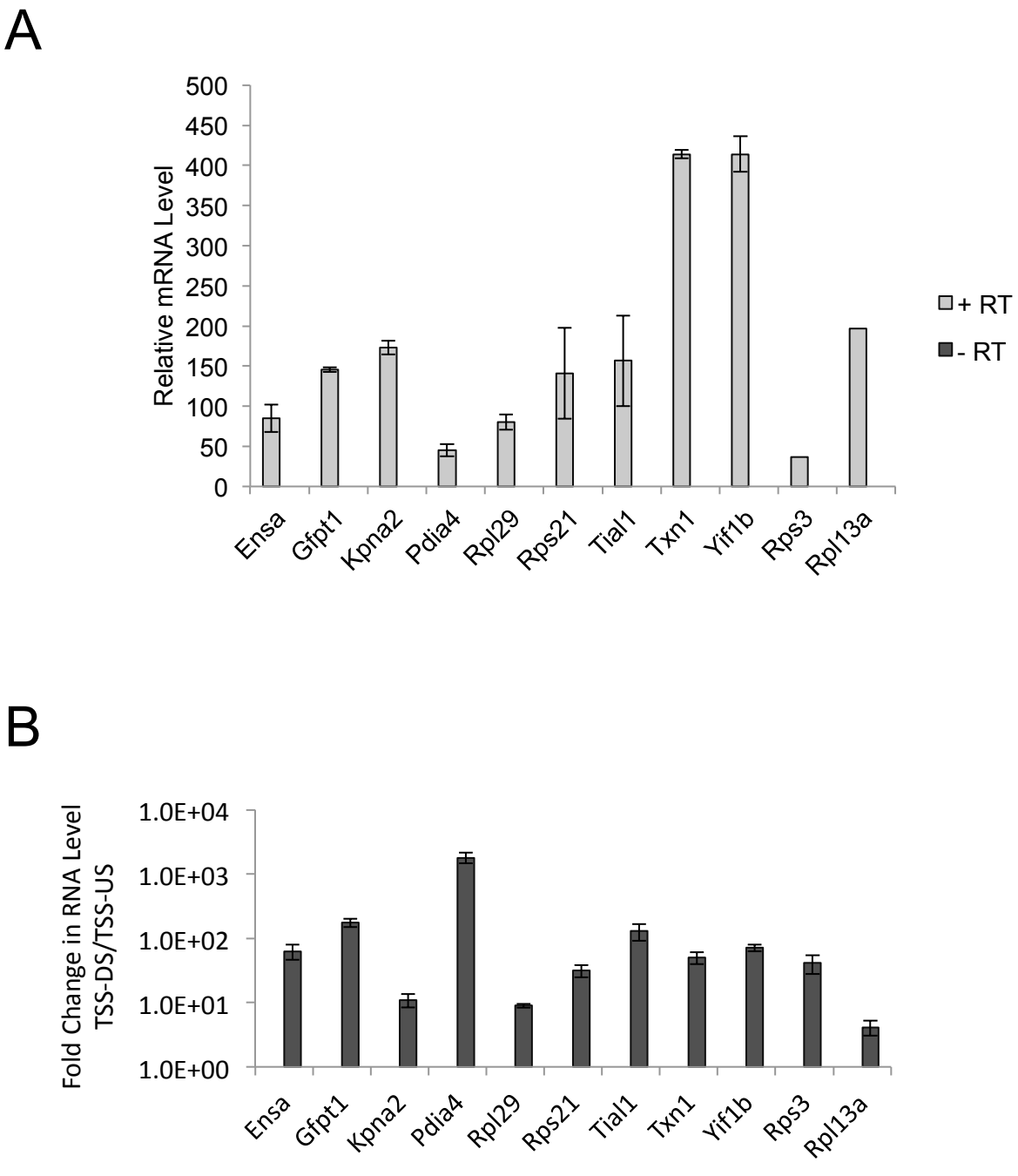

Figure S5. Characterization of TSS-US RNA. (A) Confirmation of the RNA-specific signal in qRT-PCR. cDNA was prepared from total RNA samples with random primers in the presence (+ RT) or absence (- RT) of reverse transcriptase. Real-time PCR was carried out with primers located in TSS-US of the corresponding genes. The level of 18s rRNA was used for normalization. In each case, the level of the "-RT" signal was set as one. The result indicates that the signals detected in the +RT samples are unlikely due to genomic DNA contamination in the RNA samples. (B) Comparison of RNA levels between the TSS-US and -DS regions. RNA levels in TSS-US and -DS regions were determined in the same manner as (A), in the presence of reverse transcriptase. The ratio between TSS-DS and -US levels is presented here. Results shown here are average of three experiments. Error bar: s.e.m.

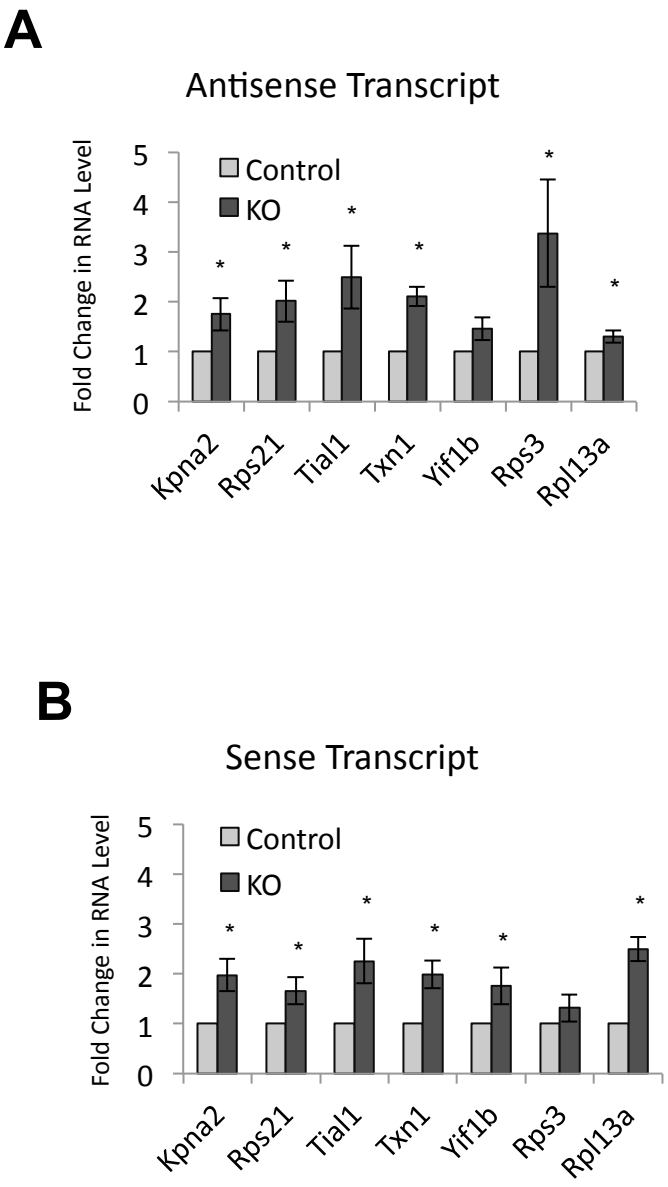

Figure S6. Further characterization of Nelf-b effect on TSS-US transcription. Effects of *Nelf-b* KO on the levels of sense (A) and anti-sense (B) transcripts upstream of TSS (TSS-US) were assessed by strand-specific RT-PCR. Primer sequences and detailed position information are available in Table S5. At each locus tested, the level in control cells was set as 1. Results shown here were average of six experiments. Error bar: s.e.m. Asterisk: $p<0.05$.