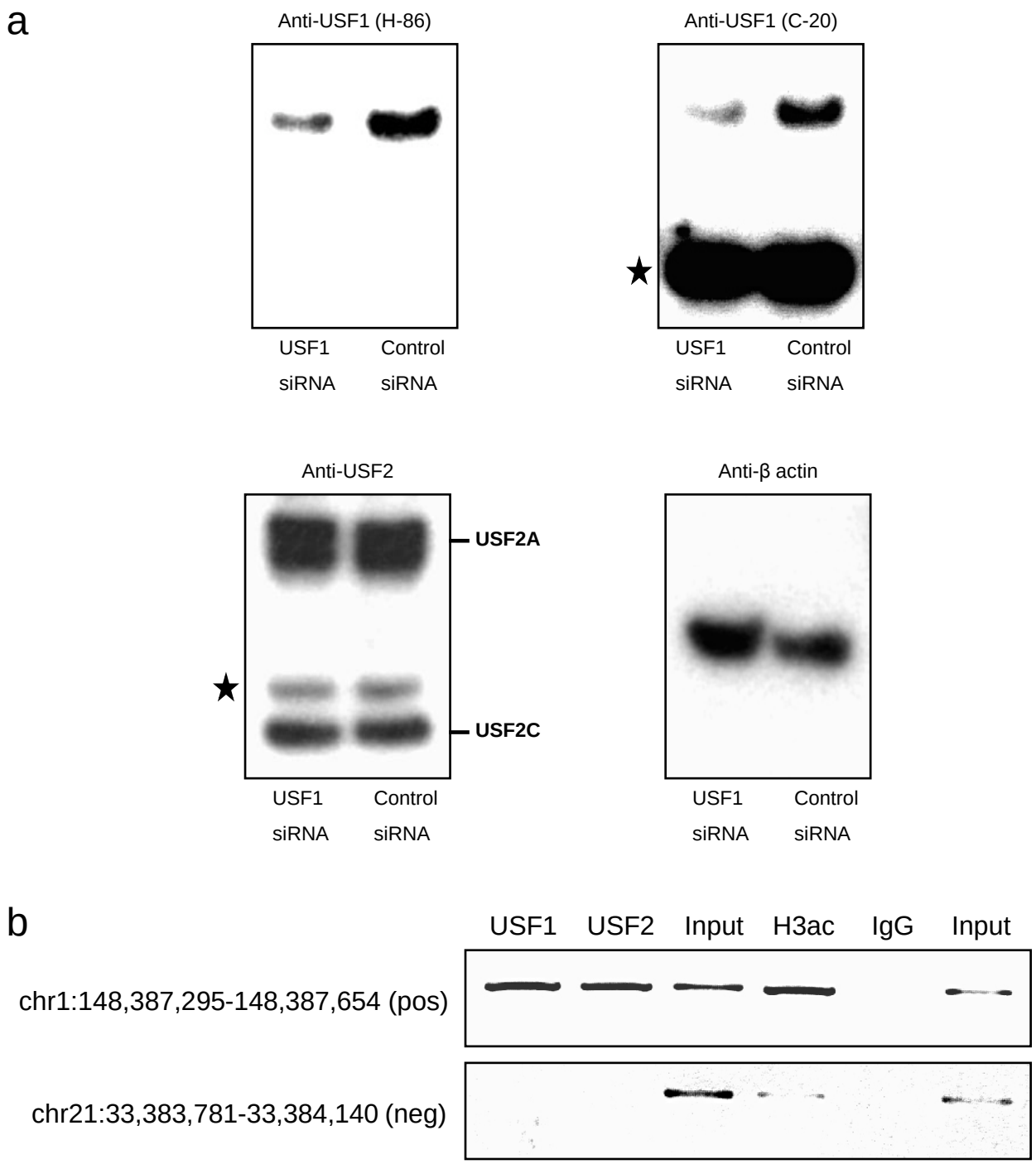
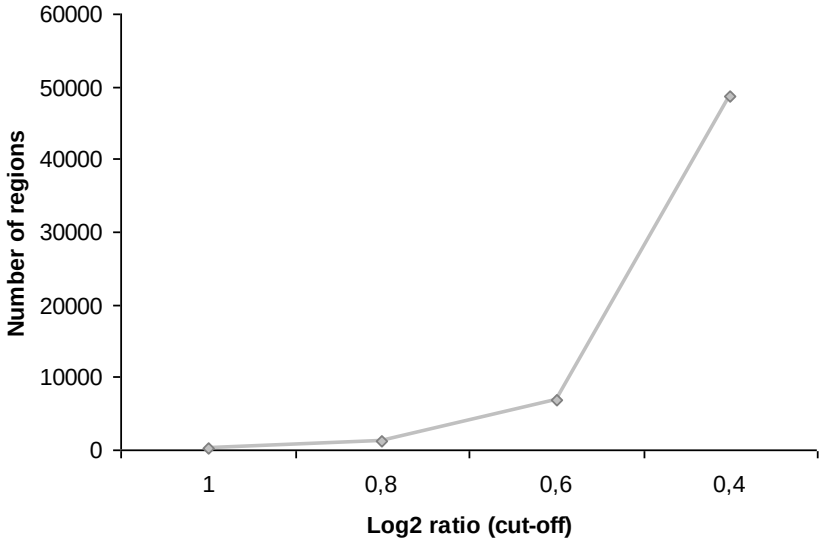
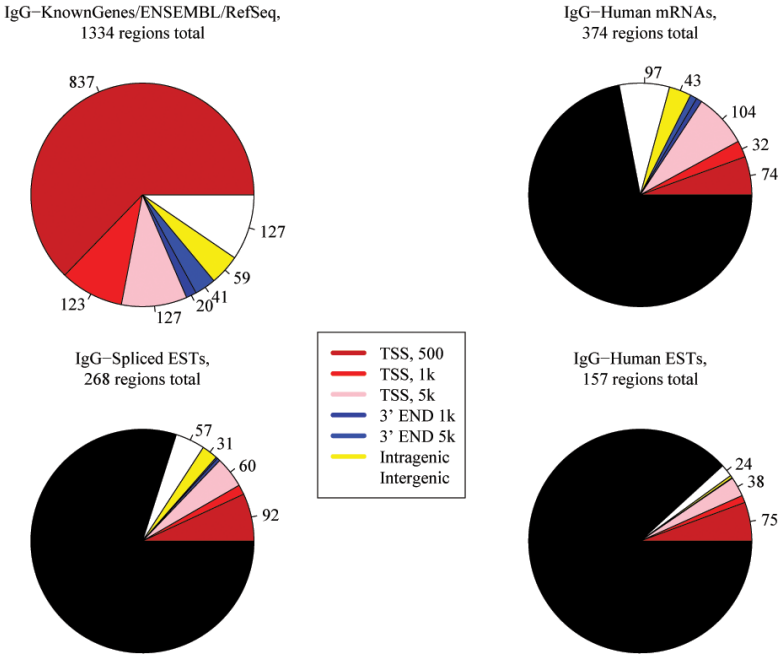


Supplementary Figure 1. Specificity and ChIP suitability of employed antibodies.
(a) Western-blot analysis of HepG2 nuclear extracts obtained after using USF1 siRNA or control siRNA. NE and CE represent nuclear and cytoplasmic extracts respectively, while the star indicates bands of unknown specificity/origin. The unspecific band detected with USF1 C-20 antibody is slightly smaller than the reported size of USF1-BD isoform, which theoretically should be detected by USF1 H-86 antibody as well. (b) ChIPs against USF1 (H-86) and USF2 were prepared in parallel, sharing the same input material. H3ac and IgG ChIPs were similarly performed. Three completely independent biological replicates were performed for each antibody, and we typically verified the success of the immunoprecipitation by PCR analysis of positive (pos) and negative (neg) control regions.

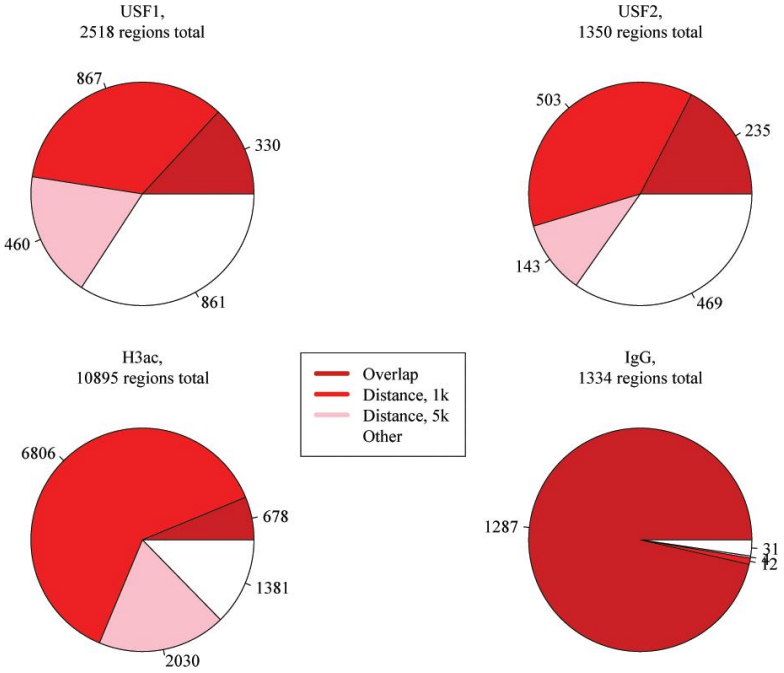
a



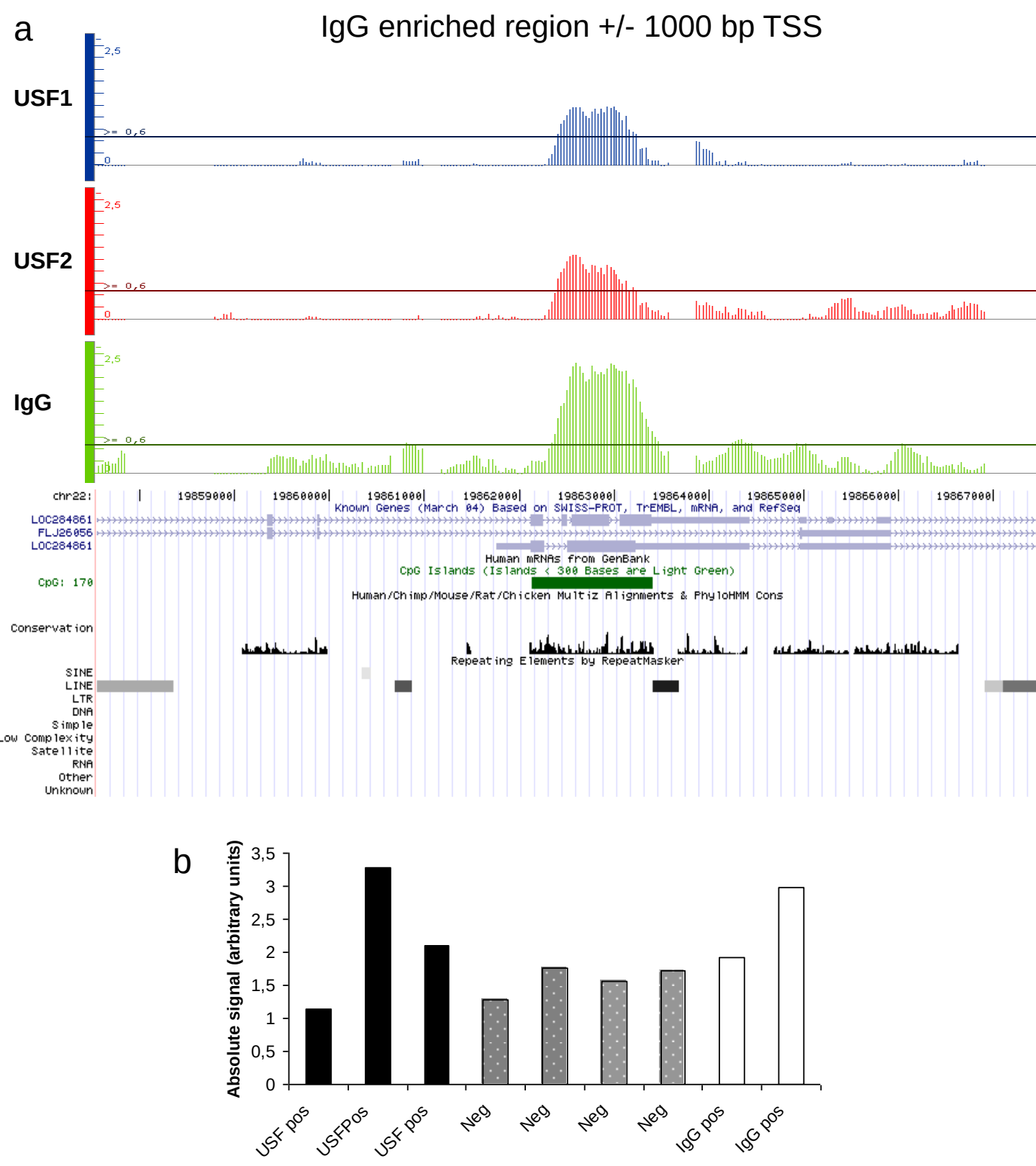
b



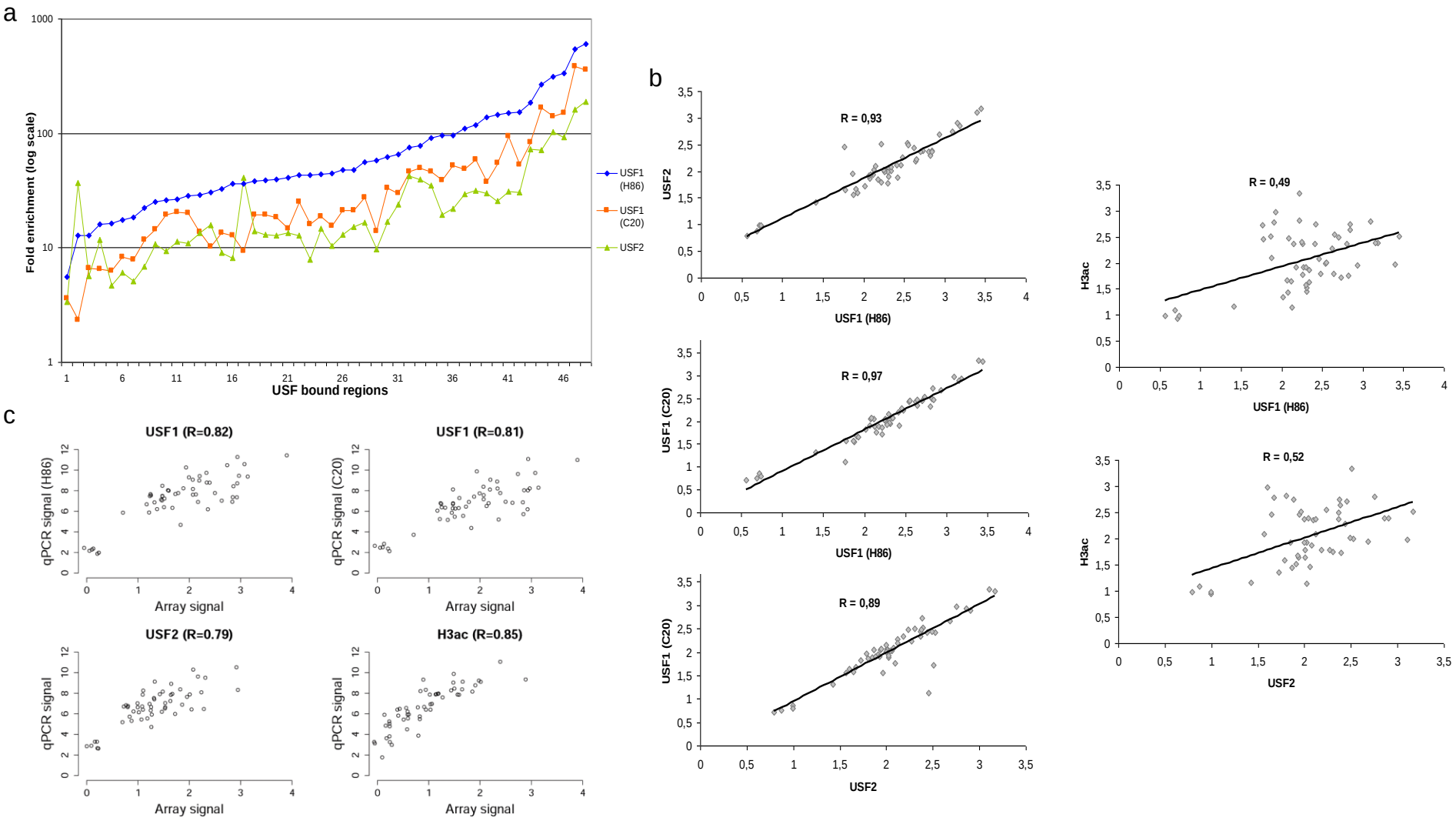
c



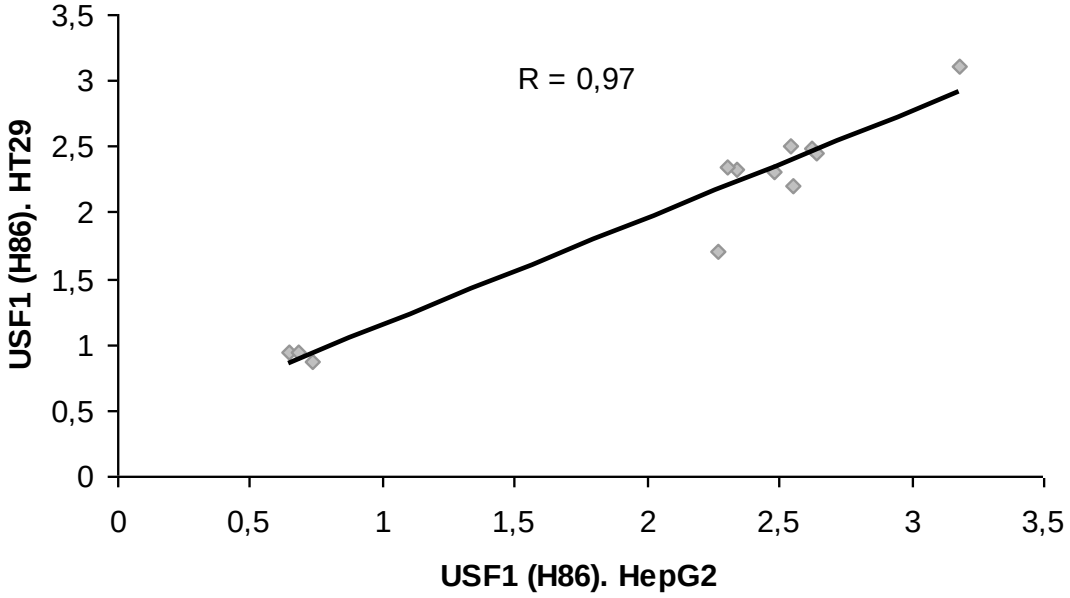
Supplementary Figure 2. Importance of IgG controls in order to reduce false positives.
(a) Number of regions that where at least 150 bp in length and display enrichment levels higher than the indicated log2 values in IgG array experiments is presented. (b) Regions with enrichment levels higher than log2=0,8 for IgG experiments were mapped to different genomic annotations in a hierarchical manner. (c) Regions with enrichment levels higher than log2=0,8 for IgG experiments were mapped to CpG islands considering the different distances indicated by the colours. Pie charts are also presented when considering USF1, USF2 and H3ac stringent data sets.

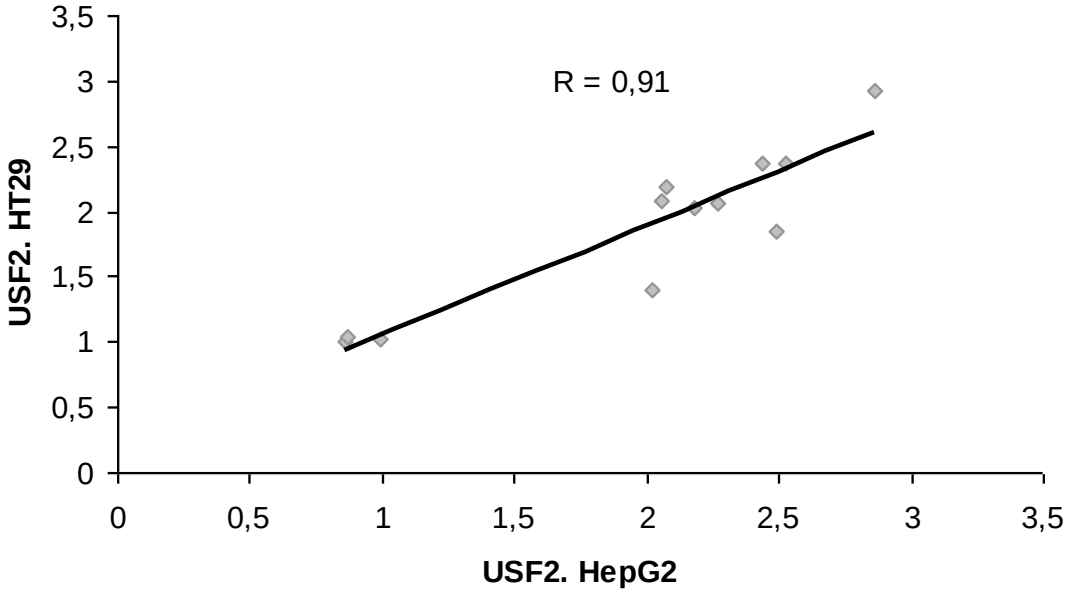


Supplementary Figure 3. Characteristics of IgG enriched regions.
(a) Example of region displaying high signal (log2 scale) for IgG ChIP-chip experiments is presented, together with the corresponding signals in USF1 and USF2 ChIP-chips. (b) qPCRs using IgG ChIP DNA were performed, analyzing regions being positive for USFs, for IgG or negative for both. Similar low levels of enrichment were observed in all cases.

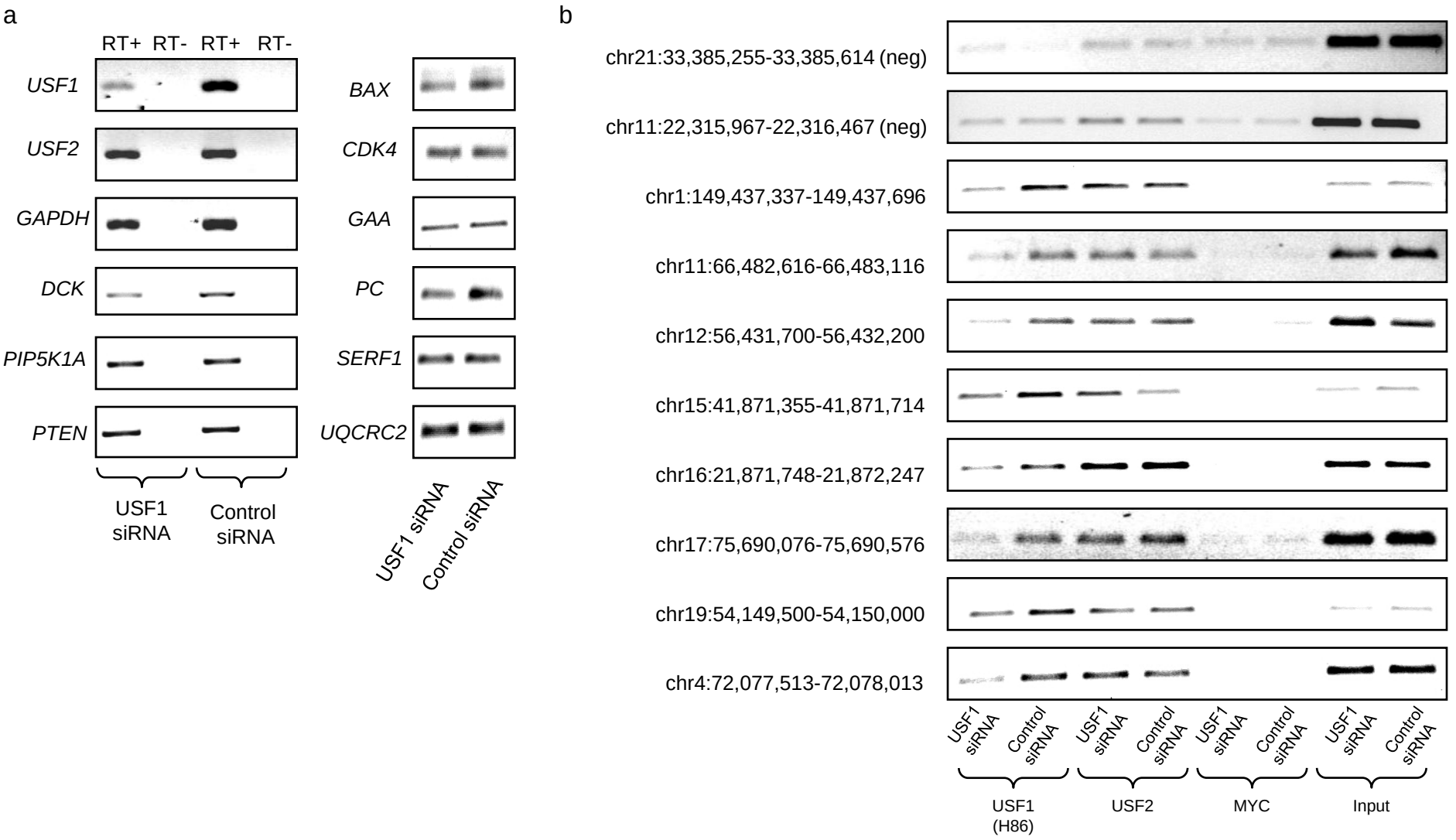


Supplementary Figure 4. ChIP-chip quality controls.
(a) 54 regions, including 6 negative and 48 positive for USF proteins were analyzed by qPCR using newly performed ChIPs. The figure represents the fold enrichment of each of the 48 positive regions, when dividing the absolute signal of each region by the mean signal of all 6 negative regions. The 48 regions were ranked according to USF H-86 fold-enrichments and the Y-axes is in logarithmic scale. (b) Correlation between the 54 qPCR signals is presented between USF1 (H-86), USF1 (C-20), USF2 and H3ac in pair-wise comparisons, using log10 transformed absolute signals. (c) The array signal (X-axes) for the 54 qPCR analyzed regions was compared to qPCR signals (Y-axes), and the correlation between both types of signal was calculated using log2 ChIP DNA/Input DNA array signals and log2 transformed qPCR absolute signals. The numbers on top of each plot are R Person coefficient values.





Supplementary Figure 5. USFs binding profiles in HT29 cells.
12 of the previous 54 regions, including 3 and 9 negative and positive USF regions respectively, where analyzed by qPCR using ChIP DNAs obtained in HT29 cells. The obtained absolute signals (log_{10} transformed) were compared to the signals for those same regions in HepG2 cells.



Supplementary Figure 6. USF1 transient knock-out by siRNA.
(a) siRNA transfections were performed as described under material and methods. After RNA extraction and cDNA preparation, the expression of the indicated genes was investigated by PCR. RT+ and RT- indicate if reverse transcriptase was included or not in the cDNA preparation. (b) ChIPs after siRNA transfections were performed using different antibodies as indicated. ChIP DNAs were analyzed by PCR using same amounts of DNA for all ChIPs and 1:20 dilution of input DNA.