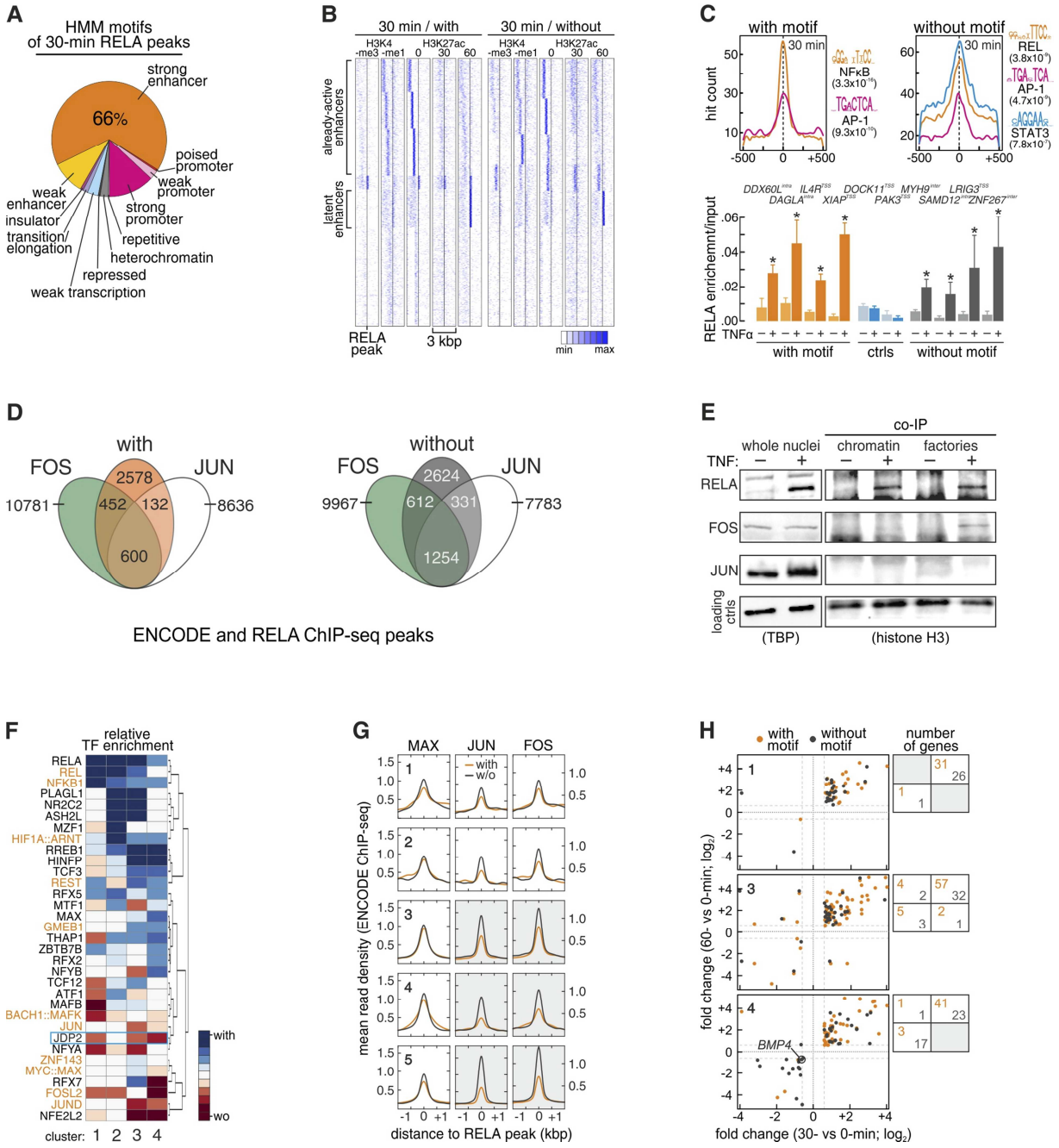

Supplemental Fig. S2. Features of sites that bind NF-κB. HUVECs were stimulated with TNF for 0, 30 or 60 min, and NF-κB (RELA subunit) binding assessed by ChIP-seq. (***A***) Most RELA peaks overlap already-active (strong or weak) enhancer regions as revealed by comparison to chromatin HMM motifs from unstimulated HUVECs (from Hoffman *et al*. 2013). (***B***) ENCODE histone marks in the 3 kbp around "with"/"without" RELA peaks. H3K4me1 and H3K27ac ChIP-seq data from HUVECs were hierarchically clustered using SeqMiner. Already-active enhancers bound by RELA and latent enhancers are indicated. (***C***) *Top*: Motif analysis at RELA peaks. Line plots show the distribution of the most-enriched motifs for ±500 bp around "with" and "without" RELA peaks. Motif logos and their respective recovery *P*-values (in parentheses) are shown next to each plot. *Bottom*: RELA ChIP-qPCR at typical sites binding the factor

that overlap a canonical NF-κB motif (*orange*) or not (*grey bars*); enrichments (±SD) at the TSS of the non-RELA-binding *DOCK11* and *PAK3* genes serve as a control. (***D***) Venn diagram shows the overlap of RELA "with" (*top*) or "without" ChIP-seq peaks (*bottom*) with FOS and JUN ENCODE ChIP-seq data. (***E***) FOS, but not JUN, co-immunoprecipitates with RELA at active sites of transcription. Soluble chromatin (detached using DNase I) and the transcriptionally-active fraction of resting (-TNF) or stimulated HUVECs (+TNF) were isolated (as described in Melnik et al. 2016), and used in co-immunoprecipitation assays. Extracts from whole nuclei were used to assess total protein titers; TBP and histone H3 levels provide loading controls. (***F***) Relative enrichment of TF-binding motifs in DNase I-hypersensitive footprints that overlap "with" or "without" RELA binding-sites (clustered as in Fig. 2G). TFs induced by TNF are colored orange. (***G***) Occupancy (in reads per million) by MAX, JUN and FOS in the 3 kbp around RELA "with" and "without" peaks (clustered as in Fig. 2G). (***H***) Log_2-fold change in intronic RNA levels (30- or 60- versus 0-min; only changes of at least ±0.6 are shown) of genes associated (within the same TAD) with RELA peaks belonging to clusters 1, 3, and 4. Genes associated to "with" or "without" RELA peaks are coloured orange and black, respectively, and their number in each quartile are shown (*right*).