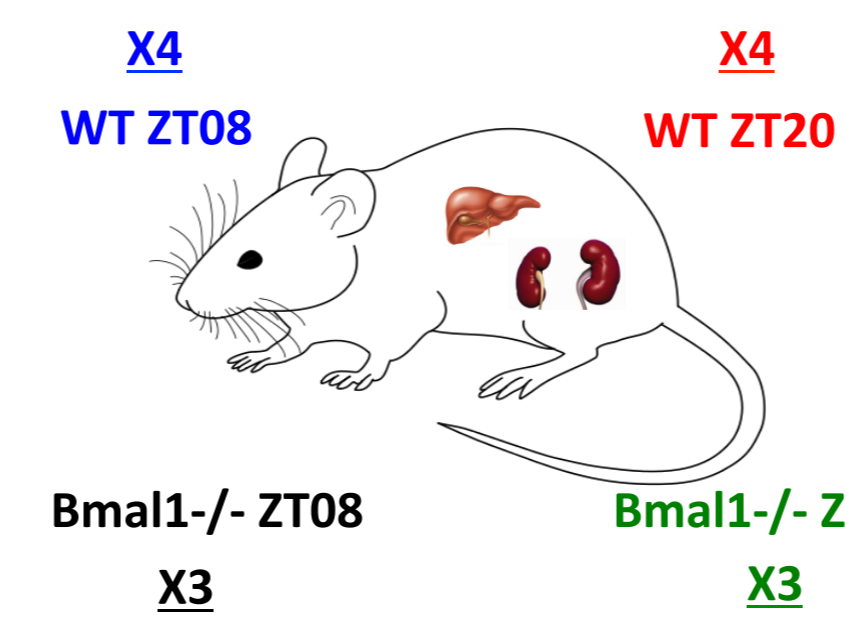
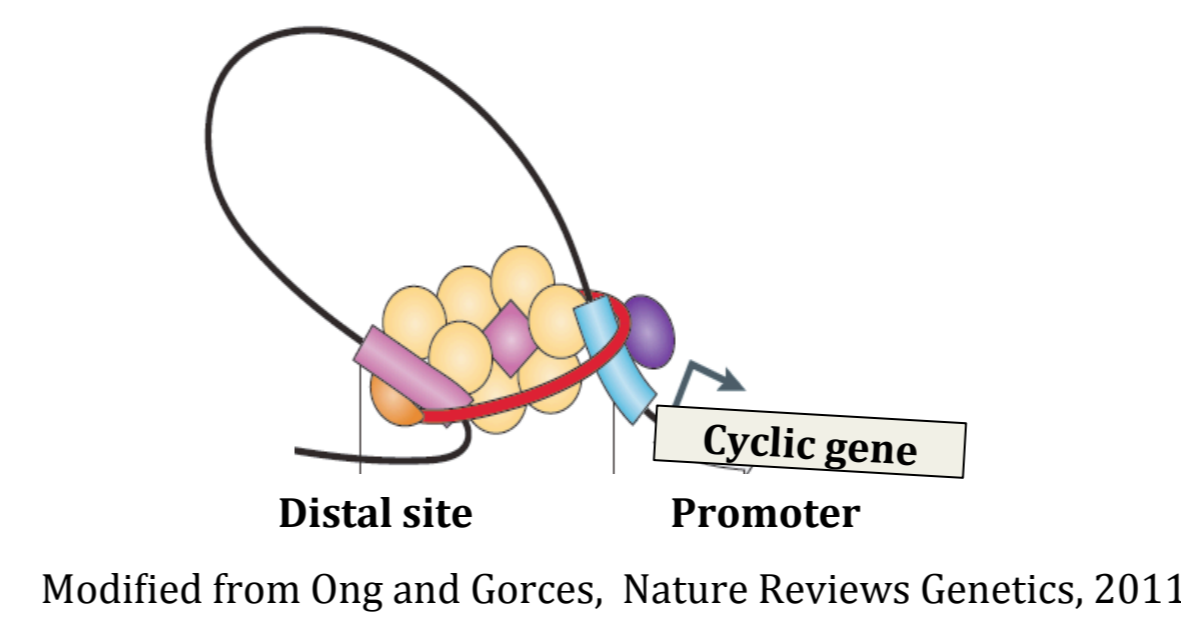


ABSTRACT

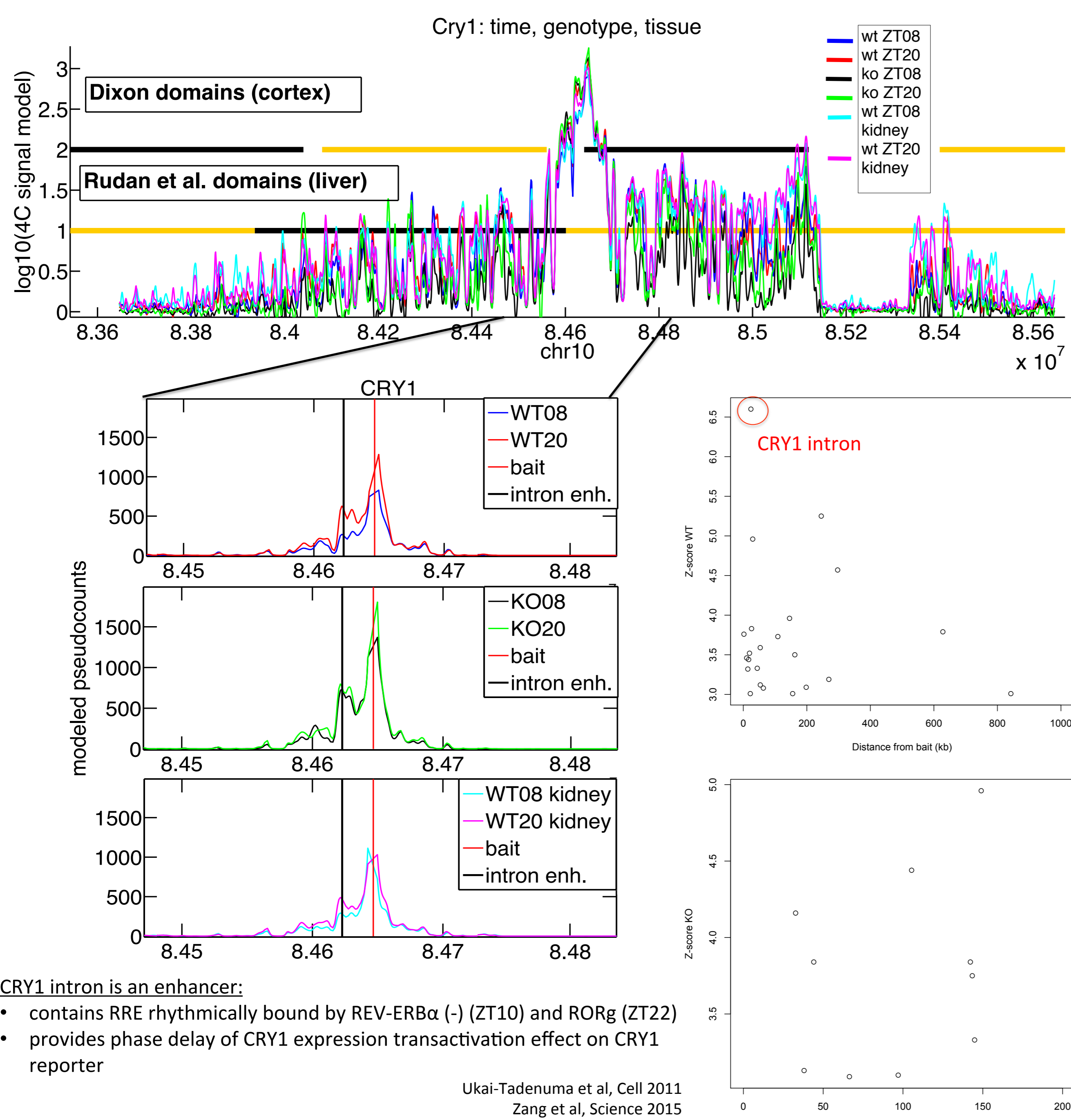
Since topological organization of chromatin plays an important role in transcription regulation and the circadian oscillator provides a unique model for dynamic gene expression, we explored the topological organization of chromatin surrounding promoters of core clock and rhythmic output genes in mouse tissues of WT and BMAL1 KO animal during the circadian cycle using 4C-sequencing (4C-seq). We found chromatin interaction patterns that are highly reproducible between biological replicates, conserved across tissues and genotypes, and gene specific. Nevertheless, we identified time-varying chromatin reorganization and DNA loops that depend on a functional molecular clock and correlate with transcription. As a highlight, we identified a robust, conserved across tissues and rhythmic interaction between the *Cry1* promoter and its intronic enhancer that depends on a functional molecular clock, suggesting that regulation of chromatin topology by the circadian clock is a regulatory layer for transcription control.

QUESTION, METHOD and DESIGN

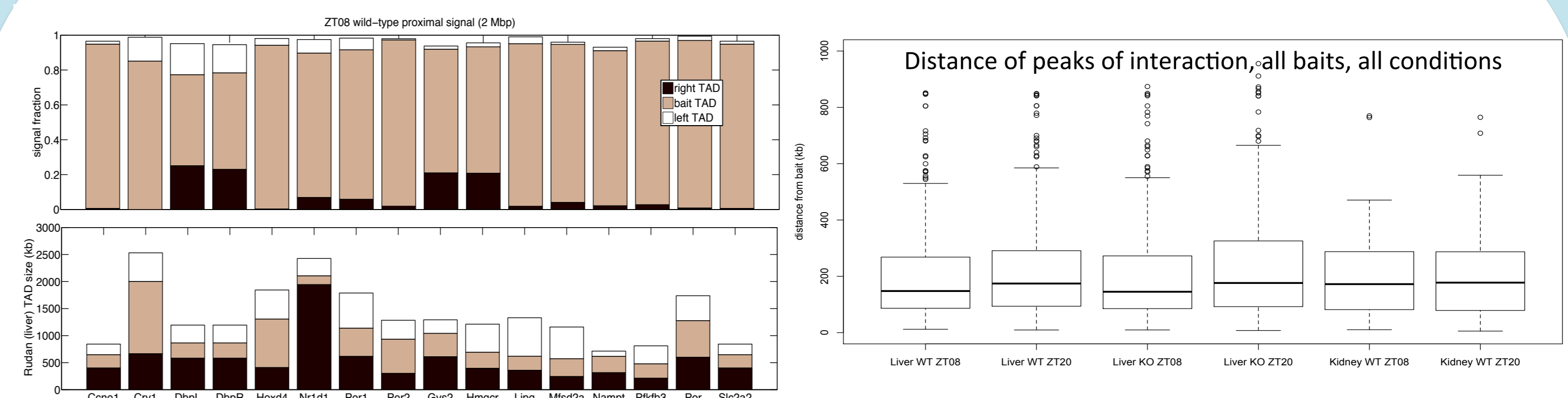


To answer if promoter of circadian genes interact with other genomic element (genes, enhancers...etc), we used 4C-seq assays that allows to reveal interactions between a single restriction fragment called the "bait" and the entire genome. We performed 4C-seq in mouse liver and kidney of WT and BMAL1 deficient mice. Therefore, we could evaluate the dynamic, tissue specific and genotype specific genomic interaction profiles of our selected baits.

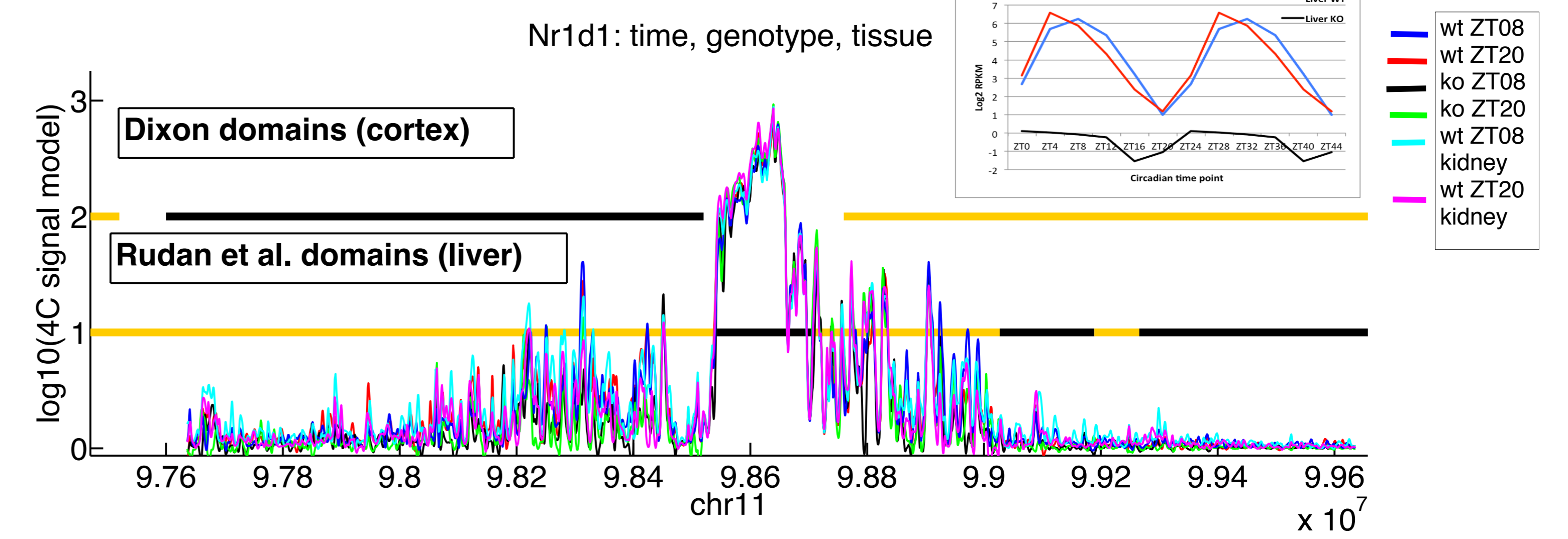
4C-seq REVEALS CLOCK DEPENDENT INTERACTIONS



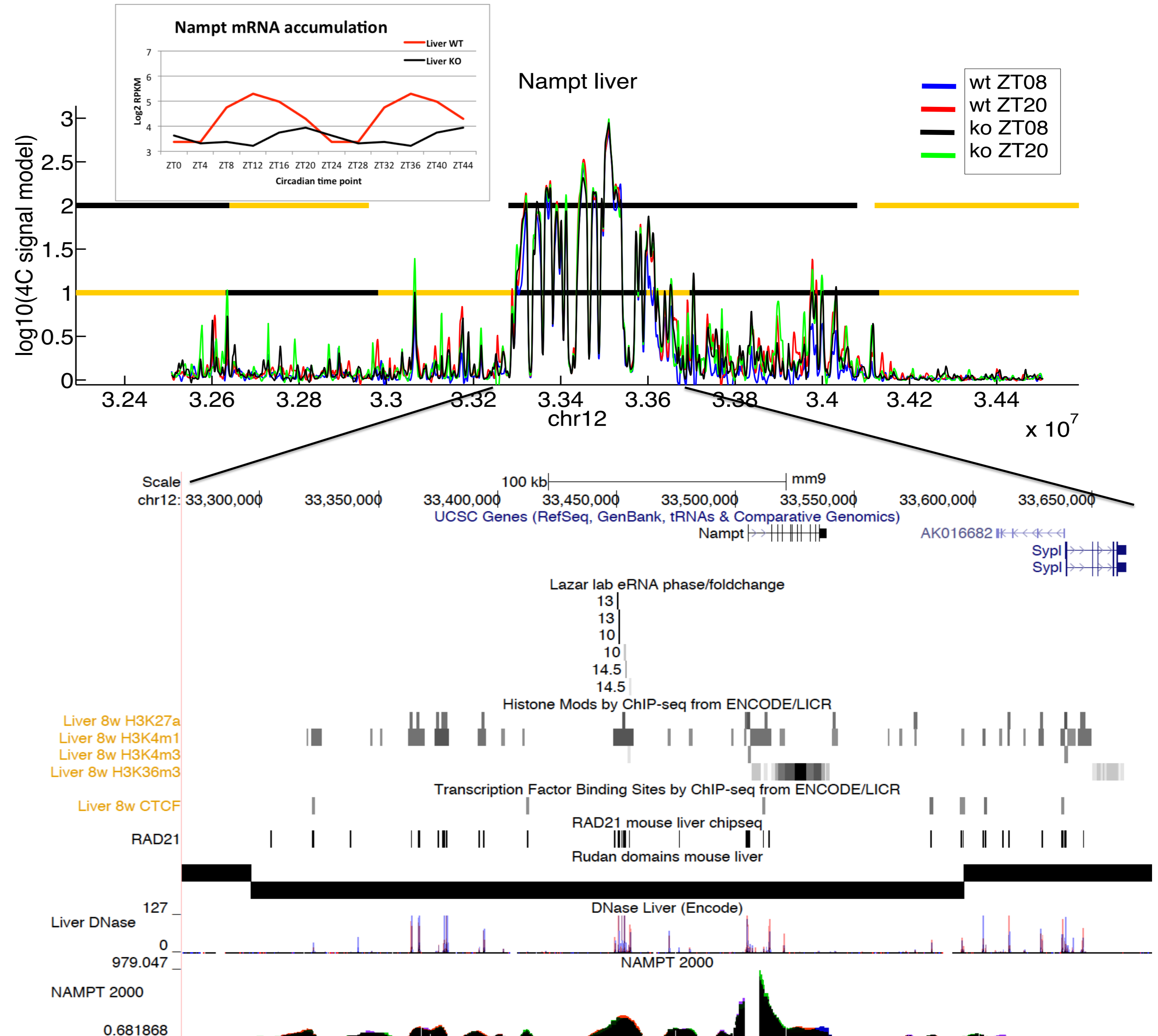
GENOMIC INTERACTIONS ARE LOCALIZED NEAR BAITS



4C-seq PATTERNS ARE CONSERVED ACROSS CIRCADIAN TIME POINTS, GENOTYPES AND TISSUES.



GENOMIC INTERACTIONS ARE ENRICHED FOR ACTIVE CHROMATIN MARKS.



CONCLUSIONS

Our study of **chromatin structure** in the context of the **circadian clock** for **multiple tissues in mouse** reveals the **general stability of contact profiles within 1 - 2 Mb** of 4C baits. This observation is consistent with the typical size of contact domains, and in most cases, precisely consistent with domain boundaries measured recently in mouse liver. Nevertheless, we find some **exceptions** to the general conservation of contacts, notably at a known regulatory site (ROR/REVERB-response element) in the **first intron of Cry1**, where we observed a **rhythmic and clock dependent chromatin loop**. Finally, we observed the tendency for contacts to associate with oscillating eRNAs and active chromatin marks, indicating that circadian gene regulation may occur due to the time-dependent recruitment of transcription factors on a stable scaffold of interconnected chromatin.

4C-seq profiles for Nr1d1 (top panel) and Nampt (bottom panel) genes show a robust conservation of genomic interactions pattern between circadian time point, genotype (Nr1d1 & Nampt), and tissues (Nr1d1), independently of their transcription status. Moreover, we observed that most of the significant peaks are contained in the bait TAD. Finally, we observed a strong enrichment of active chromatin marks under 4C-seq peaks such as DNase1 Hypersensitive sites, enhancer RNAs, enhancer associated histone modification (H3K4me1, H3K27ac).