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Clock mutant promotes osteoarthritis by inhibiting the acetylation of NFκB

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Introduction

1.The core circadian clock consists of many regulatory factors such as CLOCK and BMAL1, which form a heterodimer and contribute to the activation of downstream genes.

2.the CLOCK pro_x0002_tein possesses intrinsic histone acetyltransferase (HAT) activity and the ability to acetylate a nonhistone substrate.

Introduction

3.Acetylation is one of the key mechanisms in many inflammatory diseases.

4.the expression of the NFkB protein level showns a rhythmic pattern, which means that NFkB may be regulated by circadian genes.

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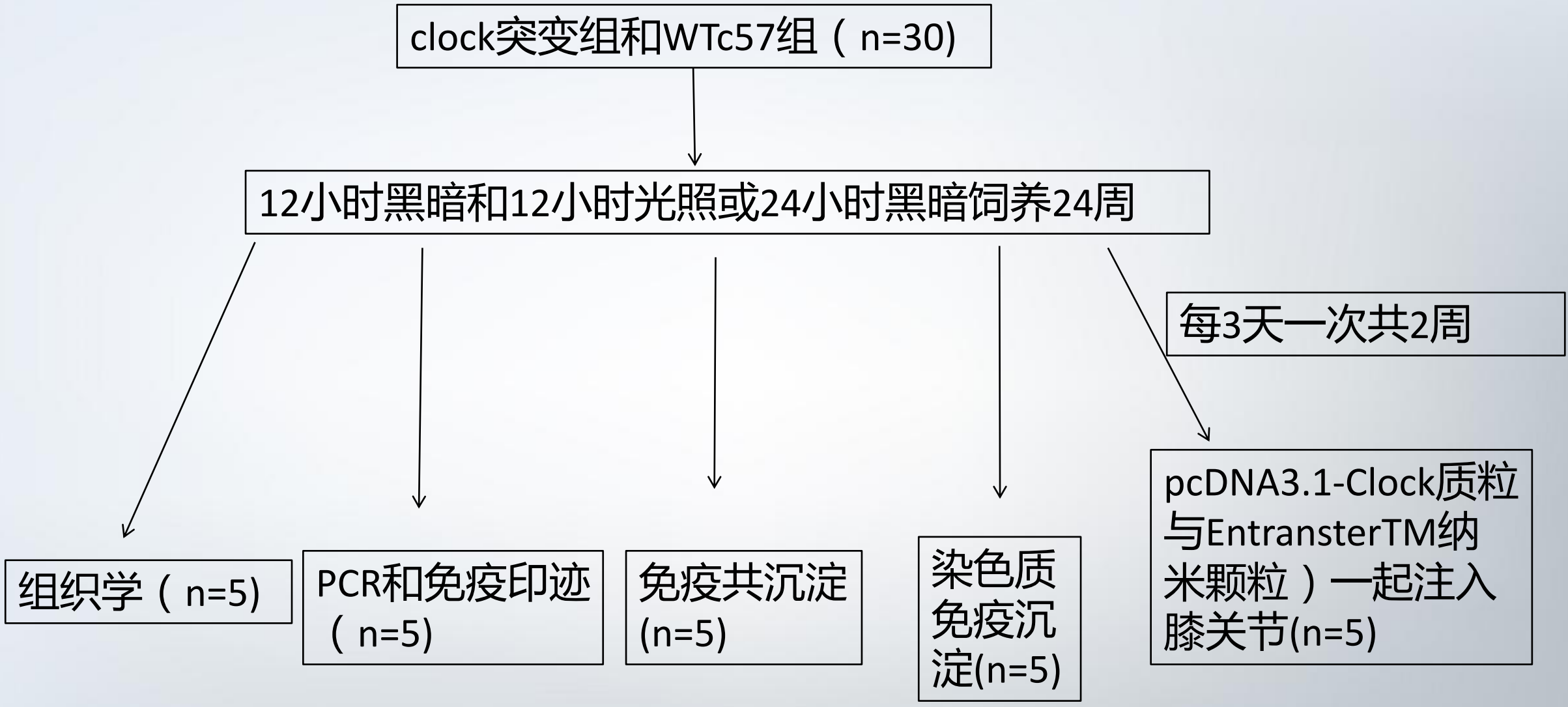
Objectives

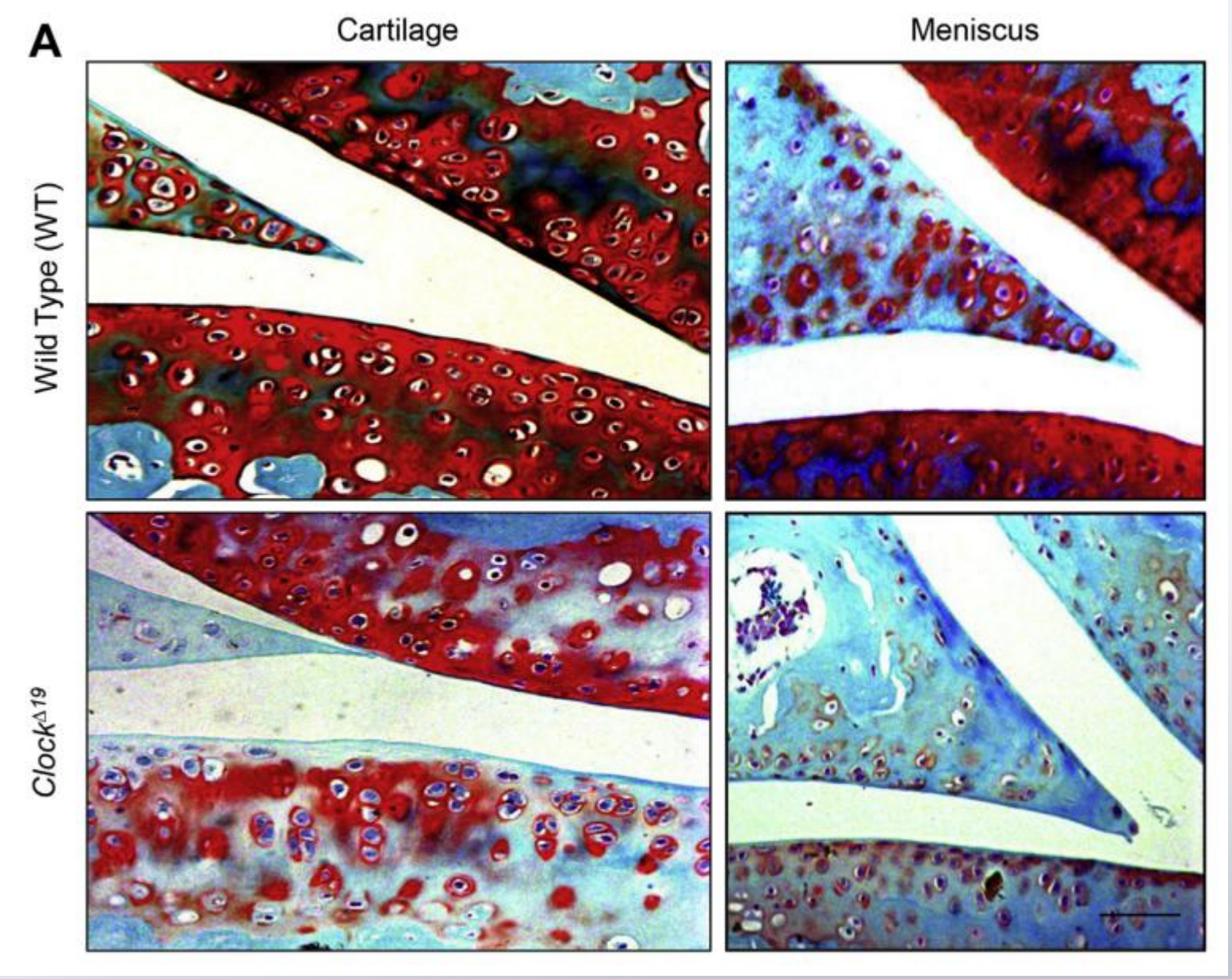
To examine the effect of the circadian gene Clock on posttranscriptional function and pro inflammatory mechanisms in osteoarthritis (OA).

Methods

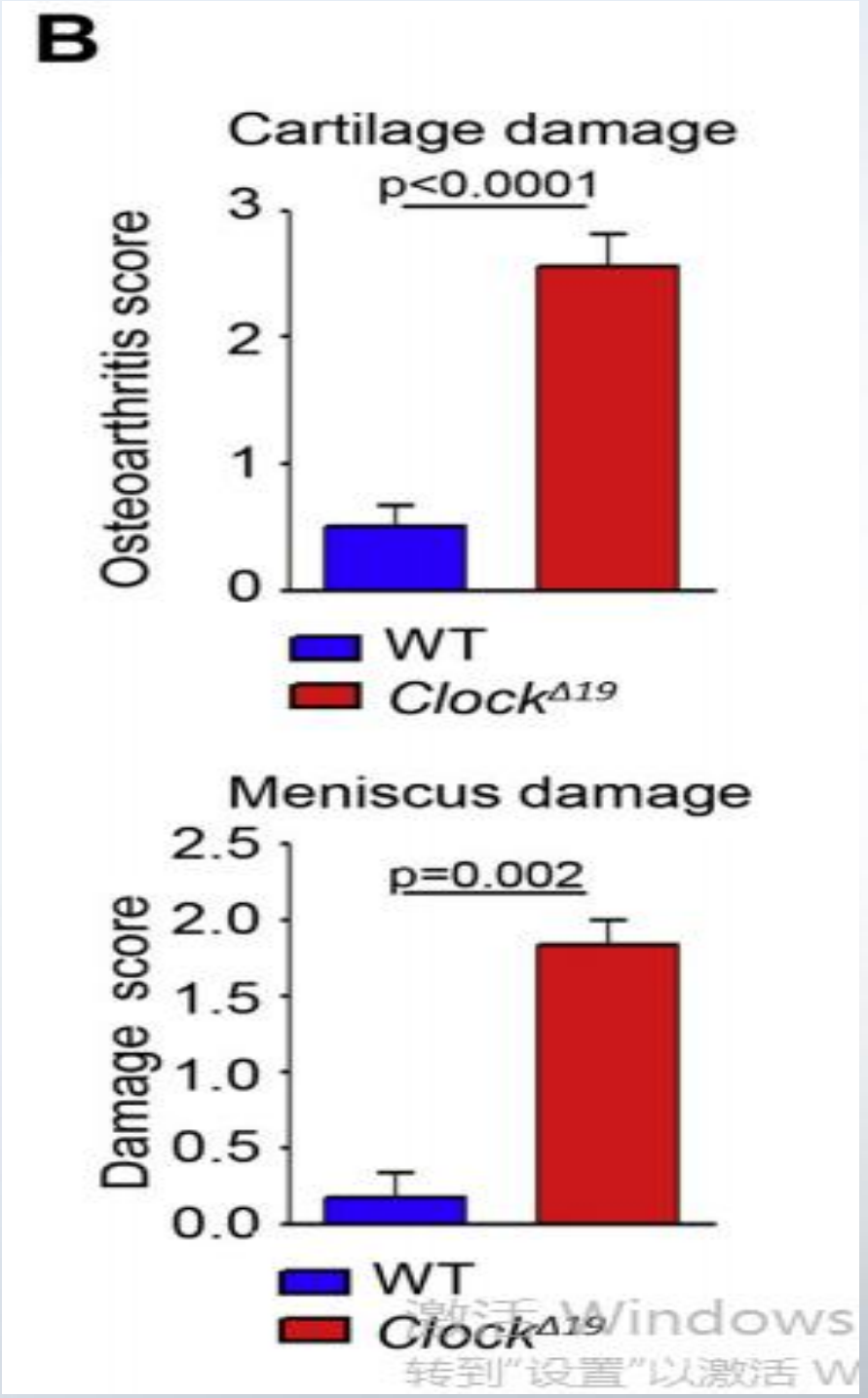
The Clock mutant (ClockD19) mice and age-matched C57BL/6J(wild type (WT)mice.


clock突变组和WTc57组（ n=30)
12小时黑暗和12小时光照或24小时黑暗饲养24周
每3天一次共2周
组织学（ n=5)
PCR和免疫印迹（ n=5)
免疫共沉淀(n=5)
染色质免疫沉淀(n=5)
pcDNA3.1-Clock质粒与EntransterTM纳米颗粒）一起注入膝关节(n=5)

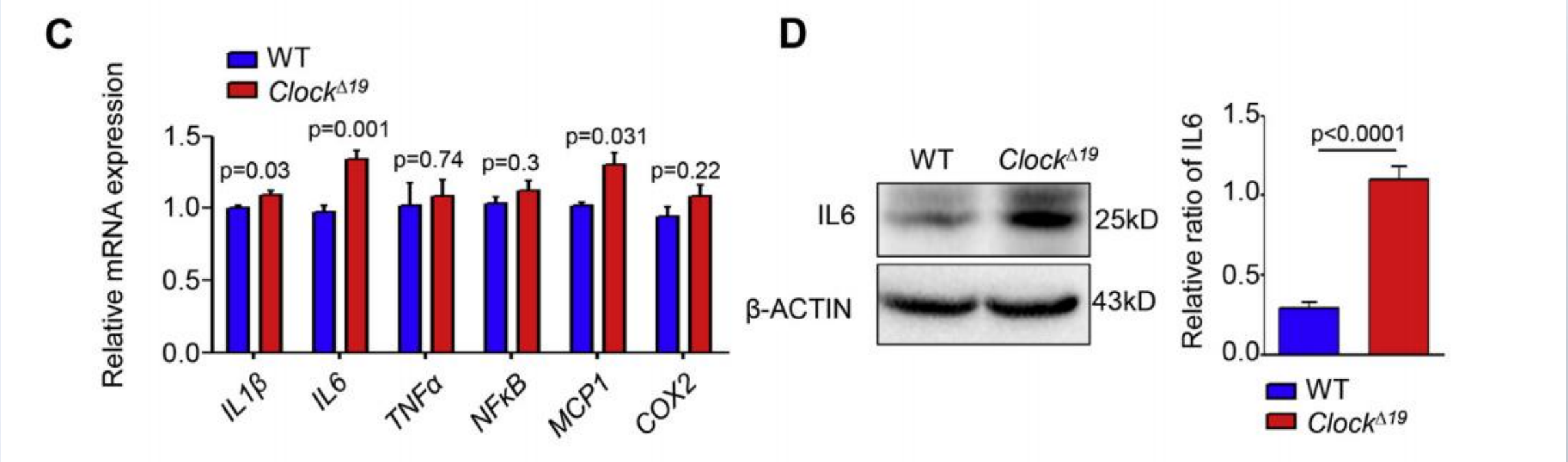
Results



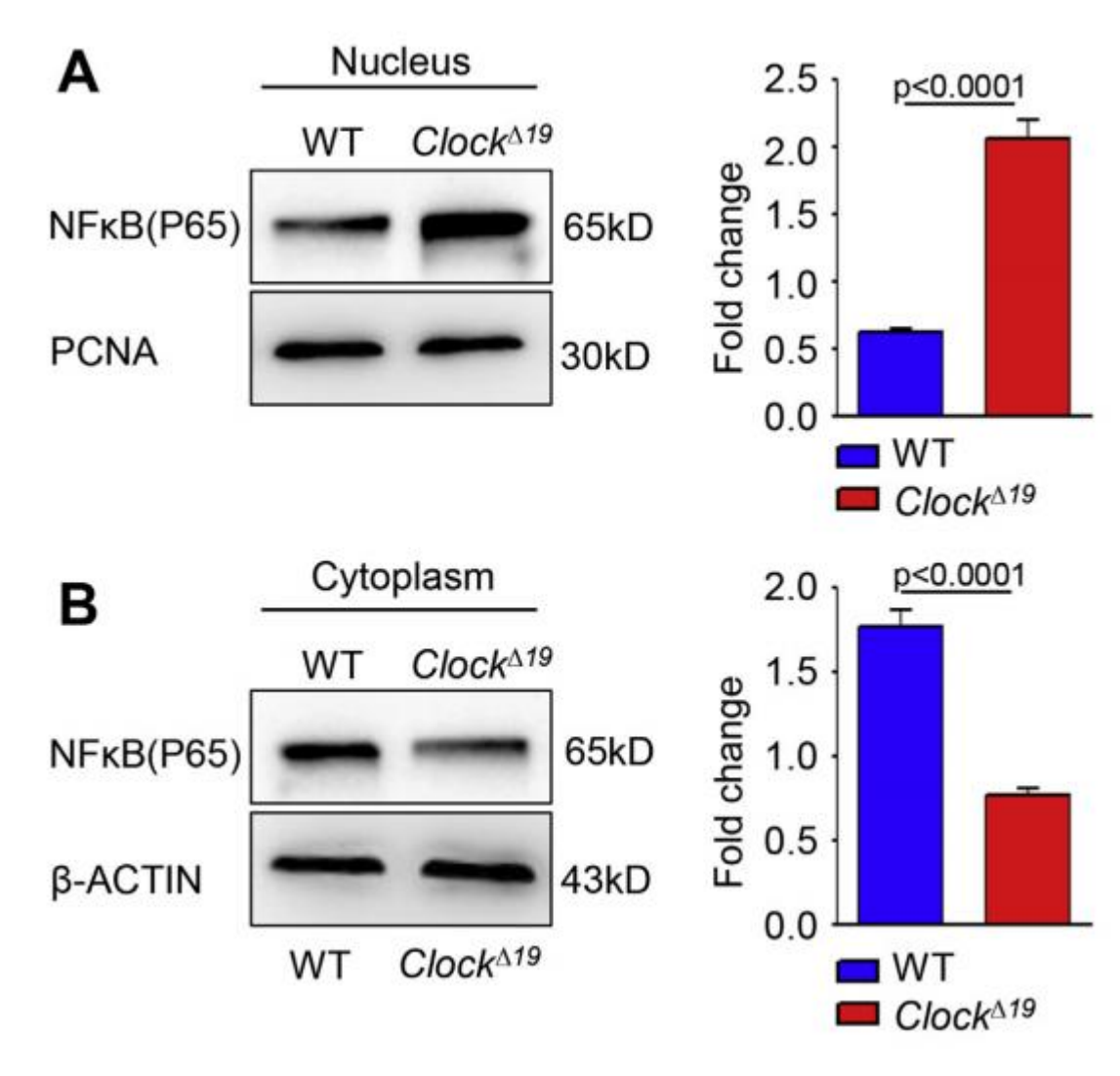
Representative histological images of wild type (WT) and Clock mutant mouse knees at ZT0. Safranin-O staining revealed cartilage and meniscus. No obvious damage to articular cartilage was observed in WT mice.



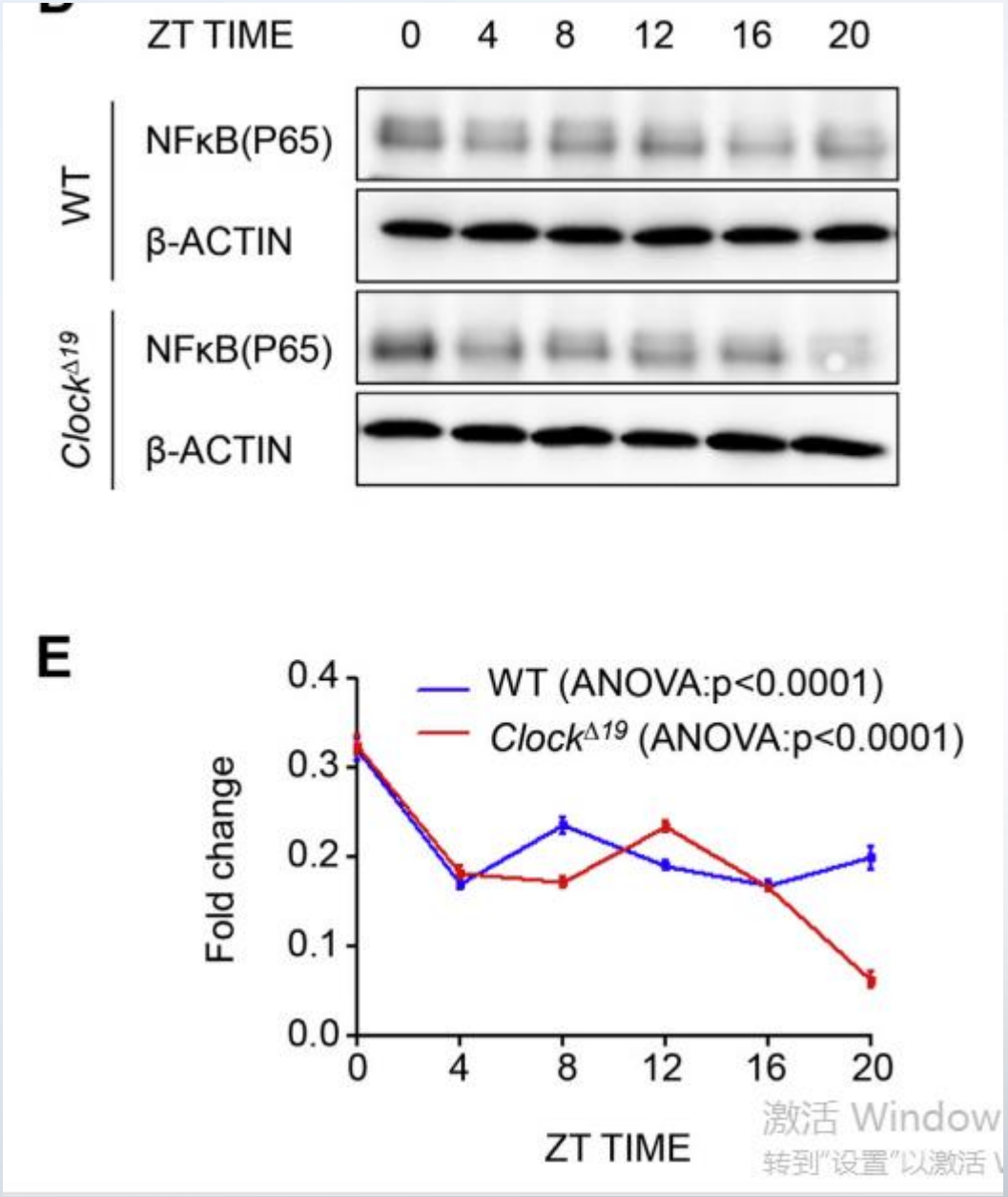
Cartilage damage and meniscus damage scores based on histology of (A).



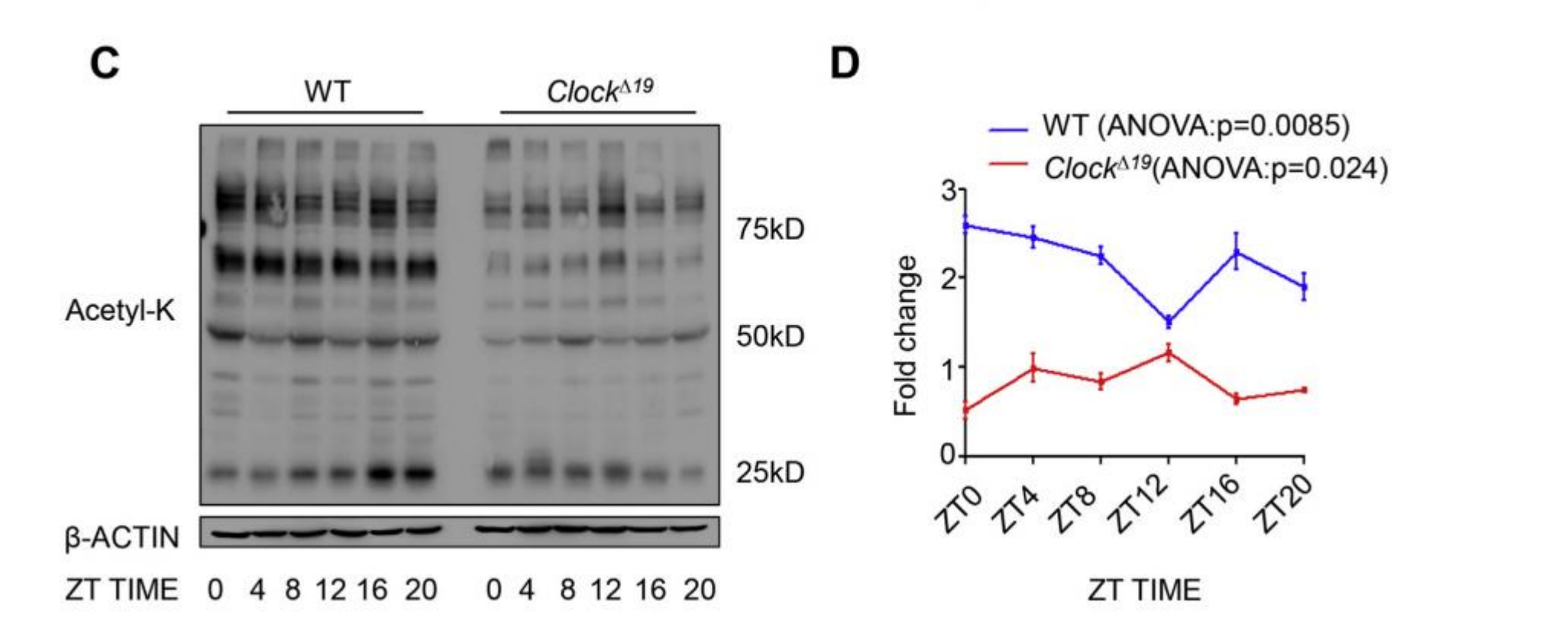
(C) Relative expression assessed by qPCR of some inflammation-related genes. Note that the genes, especially IL6, were upregulated in Clock mutant mice at ZT0. Data were normalized to Gapdh expression. (D) Immunoblots of IL6 from WT and Clock mutant mice at ZT0. Quantification of the immunoblots showed significantly increased expression of IL6. P-values less than 0.05 were considered significant



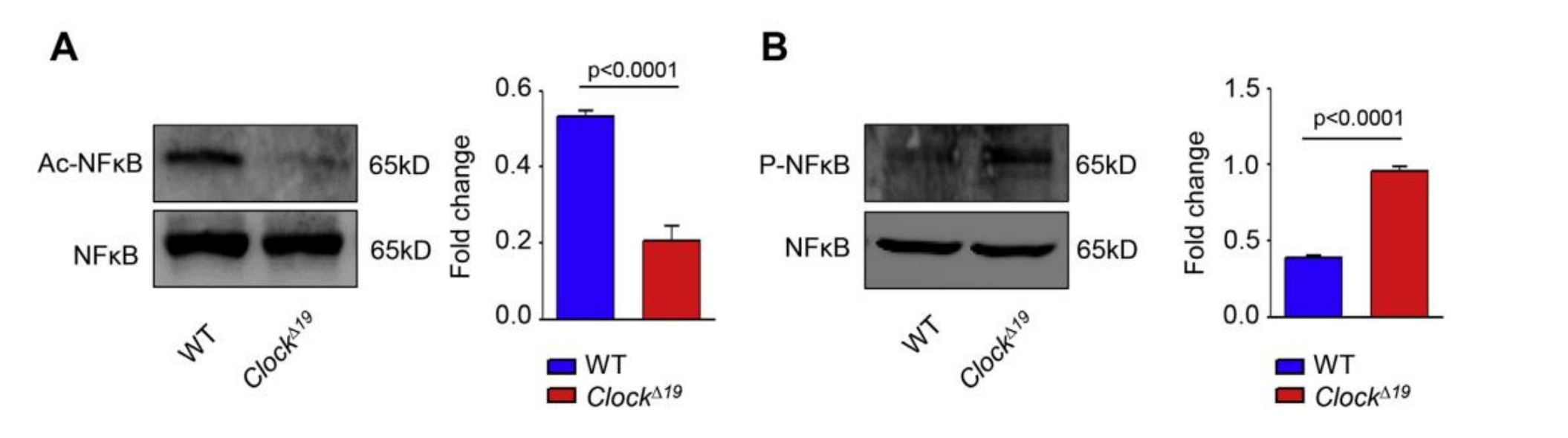
. (AeB) Immunoblots showing the expression of NFkB in the nucleus and cytoplasm of WT and Clock mutant mice at ZT0.



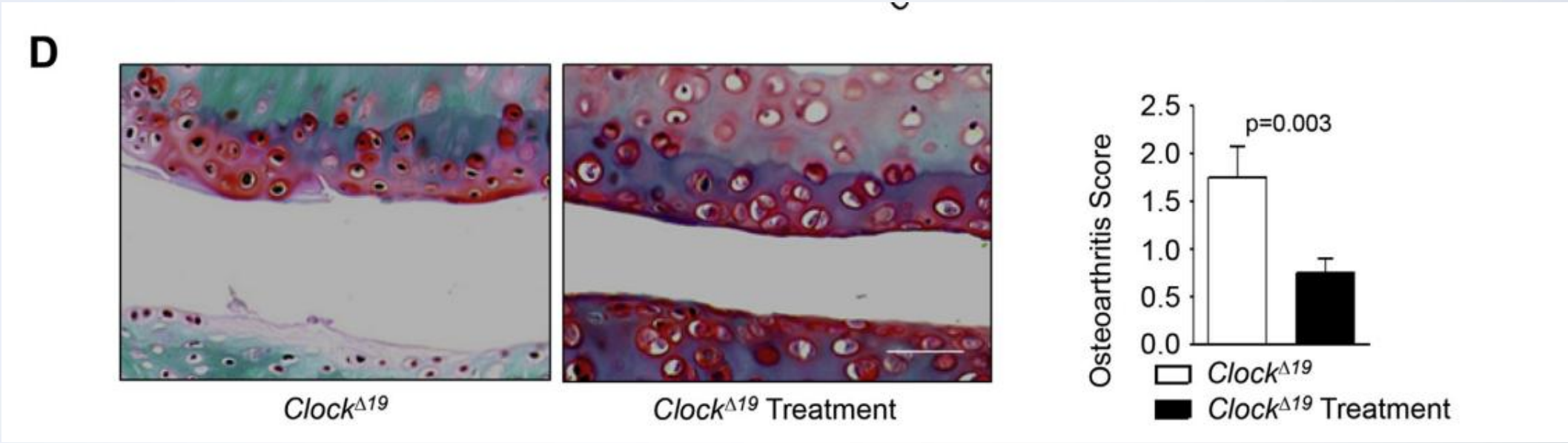
(C) Relative cytoplasmic expression of IkB was analyzed in the WT and Clock mutant mice at ZT0. (DeE) The cartilages were harvested at the indicated time points (ZT0- ZT12) in WT and Clock mutant mice. The protein expression of NFkB was examined by Western blot, and b-actin was used as a loading control. Note that both WT and Clock mutant mice showed rhythmic gene expression levels of NFkB, but no significant changes were found between the two groups. Data were analyzed by one-way ANOVA and Bonferroni post hoc tests and P-values less than 0.05 were considered significant.



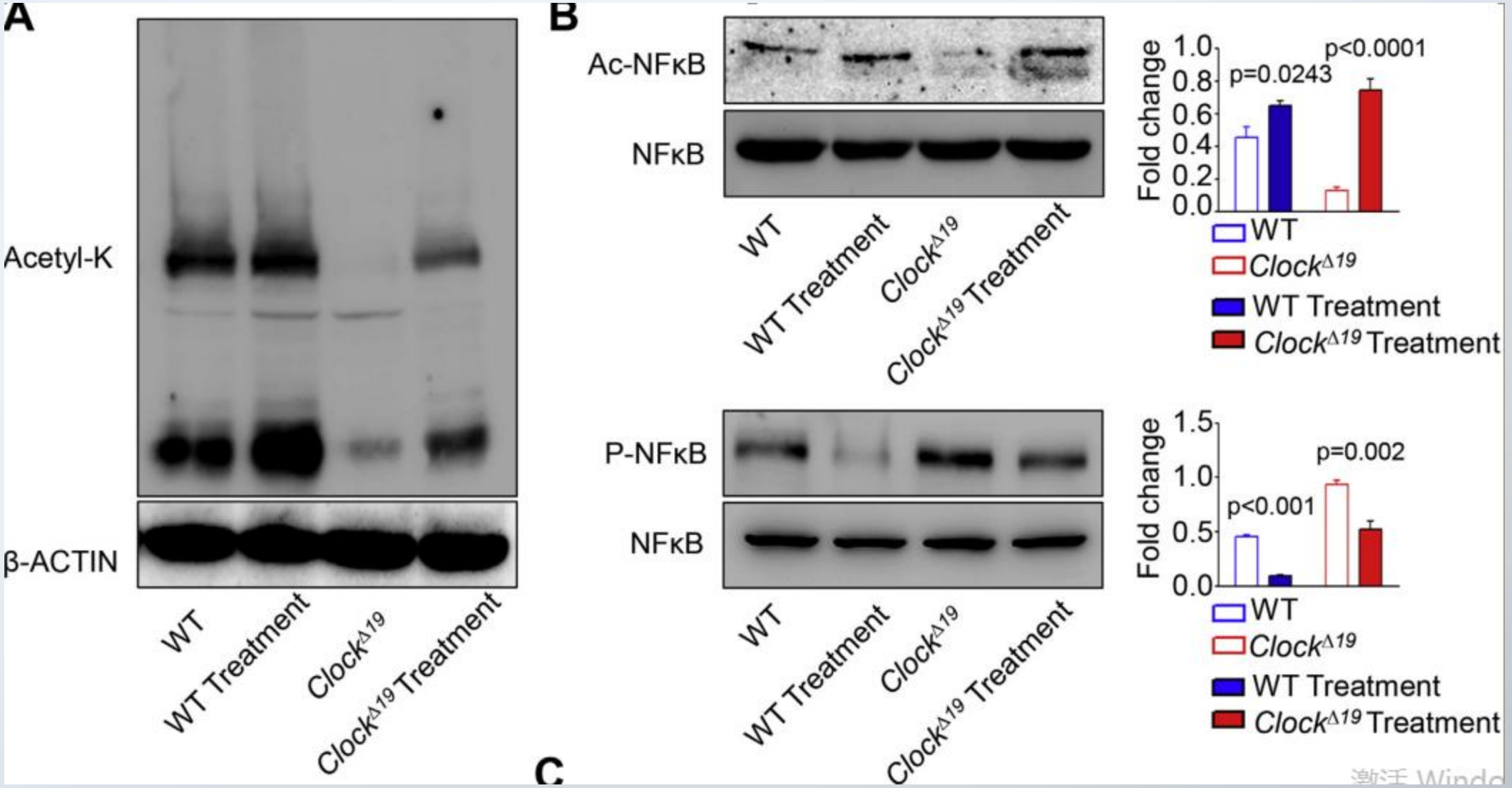
Immunoblots showing the total acetylation levels in the WT and Clock mutant mouse cartilage tissues. Note that the Clock mutation decreases the total acetylation and disrupts the pattern of acetylation. Data were analyzed by one-way ANOVA and Bonferroni post hoc tests and P-values less than 0.05 were considered significant.

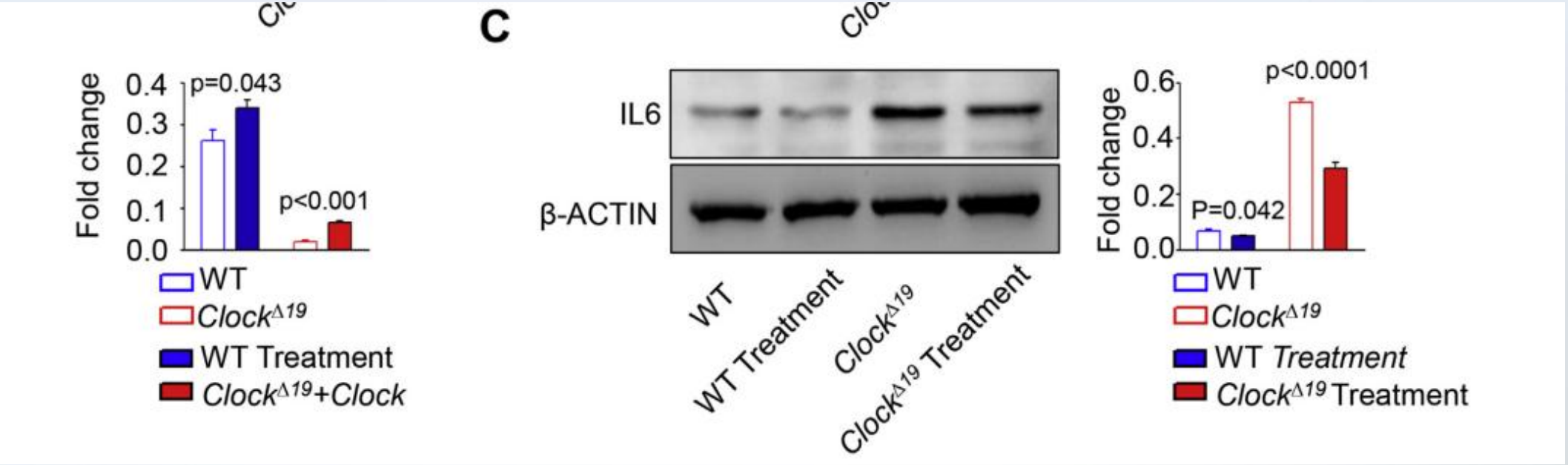


(AeB) Protein quantification of acetylated-NFkB (Ac-NFkB) and phosphorylated_x0002_NFkB (P-NFkB) in the nuclei of WT and Clock mutant mice. Decreased Ac-NFkB and increased P-NFkB are shown in Clock mutant mice. Individual protein changes are not significantly different. NFkB bands were used as an internal control. Columns in graphs show protein normalized to NFkB.

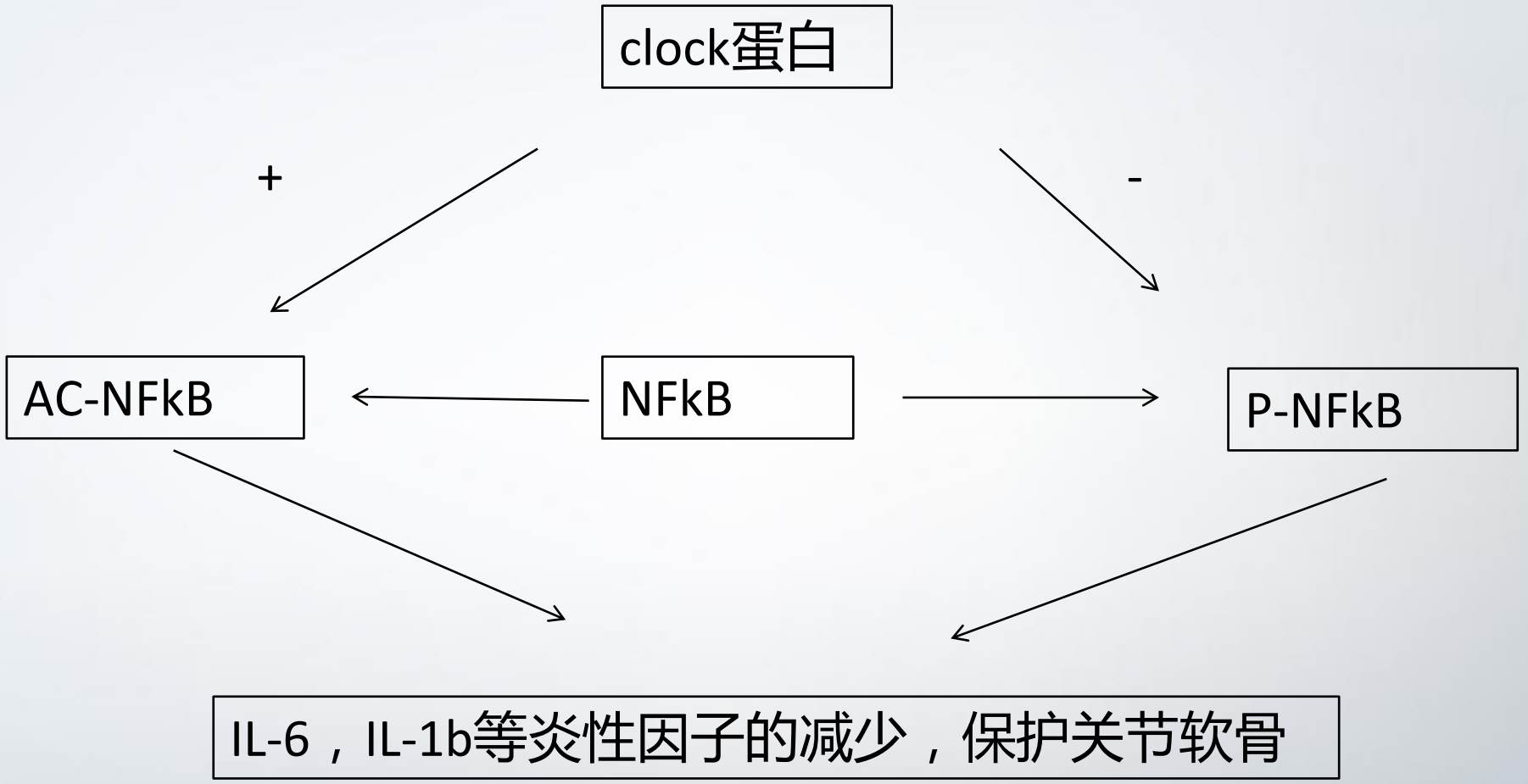


(D) Histological images of Clock mutant mice and Clock treatment (ClockD19 with Clock nanoparticles) administered to mouse knees. Cartilage damage was scored using histology images.


A
Acetyl-K
β-ACTIN
WT
WT Treatment
Clock^Δ19
Clock^Δ19 Treatment
B
Ac-NFκB
NFκB
P-NFκB
NFκB
Fold change
p=0.0243
p<0.0001
p<0.001
p=0.002
WT
Clock^Δ19
WT Treatment
Clock^Δ19 Treatment



. (A) Nanoparticle-wrapped Clock plasmids were injected into both knee joint cavities to treat osteo_x0002_arthritis in Clock mutant mice every 3 days for 2 weeks. Cartilage tissues were harvested, and total acetylation protein levels were detected by immunoblots. Note that exogenous Clock treatment promotes the total acetylation level. (B) Mice were treated as described in (A), and Ac-NFkB and P-NFkB were analyzed by Western blot.Columns in the right show protein normalized for NFkB levels in control. n ¼ 5 mice per group. P-values less than 0.05 were considered significant. (C) Cartilage samples from (A) were used to measure IL6 protein expression.


clock蛋白
+
-
AC-NFkB
NFkB
P-NFkB
IL-6，IL-1b等炎性因子的减少，保护关节软骨

Results

1.The Clock mutant leads to cartilage degradation and inflammation activation.
we believe that IL6 may play a key role in Clock mutation-induced OA.

2.CLOCK binds and promotes NFkB activation via acetylation activity. Clock, as an acetyl_x0002_transferase, regulates NFkB transcription level by protein modification. In summary, these findings suggest that Clock decreases the acetylation of NFkB to induce inflammation.

3.Exogenous clock promotes acetylation to relieve inflammation in clock mutant mice.

conclusion

the Clock mutant causes inflammation and cartilage degradation due to a reduction in acetylation and the abnormal activation of NFkB.

Discussion

Mice with clock gene disorders can present an abnormal carti_x0002_lage phenotype13. Here, we first demonstrated the post_x0002_translational regulation of the circadian gene Clock in OA. Clockcontrols P-NFkB expression through acetylation in a steady state,but the Clock mutant (lack of exon 19) loses most of its acetylation activity, which causes excessive P-NFkB activation and eventually induces a severe inflammatory response (Fig. 7). Thus, our findings may have a significant impact on the future treatment of OA.

thanks