*Journal of Integrative Agriculture* 2022, 21(6): 1740–1754

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

# HBP1 inhibits chicken preadipocyte differentiation by activating the STAT3 signaling *via* directly enhancing *JAK2* expression

**CHEN Hong-yan[1, 2, 3*], CHENG Bo-han[1, 2, 3*], MA Yan-yan[1, 2, 3], ZHANG Qi[1, 2, 3], LENG Li[1, 2, 3], WANG Shou-zhi[1, 2, 3], LI Hui[1, 2, 3]**

[1] *Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture and Rural Affairs, Harbin 150030, P.R.China*

[2] *Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang Province, Harbin 150030, P.R.China*

[3] *College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, P.R.China*

**Abstract**

Obesity presents a serious threat to human health and broiler performance. The expansion of adipose tissue is mainly regulated by the differentiation of preadipocytes. The differentiation of preadipocytes is a complex biological process regulated by a variety of transcription factors and signaling pathways. Previous studies have shown that the transcription factor HMG-box protein 1 (HBP1) can regulate the differentiation of mouse 3T3-L1 preadipocytes by activating the Wnt/β-catenin signaling pathway. However, it is unclear whether HBP1 involved in chicken preadipocyte differentiation and which signaling pathways it regulates. The aim of the current study was to explore the biological function and molecular regulatory mechanism of HBP1 in the differentiation of chicken preadipocytes. The expression patterns of chicken HBP1 in abdominal adipose tissue and during preadipocyte differentiation were analyzed by RT-qPCR and Western blot. The preadipocyte stably overexpressing HBP1 or knockout HBP1 and their control cell line were used to analyze the effect of HBP1 on preadipocyte differentiation by oil red O staining, RT-qPCR and Western blot. Cignal 45-Pathway Reporter Array was used to screen the signal pathways that HBP1 regulates in the differentiation of chicken preadipocytes. Chemical inhibitor and siRNA for signal transducer and activator of transcription 3 (STAT3) were used to analyze the effect of STAT3 on preadipocyte differentiation. The preadipocyte stably overexpressing HBP1 was transfected by the siRNA of STAT3 or treated with a chemical inhibitor of STAT3 for the rescue experiment. The results of gene expression analysis showed that the expression of HBP1 was related to abdominal fat deposition and preadipocyte differentiation in chickens. The results of function gain and loss experiments indicated that overexpression/knockout of HBP1 in chicken preadipocytes could inhibit/promote ($P$<0.05) lipid droplet deposition and the expression of adipogenesis-related genes. Mechanismlly, HBP1 activates ($P$<0.05) the signal transducer and activator of transcription 3 (STAT3) signaling pathway

Received 7 June, 2021 Accepted 19 January, 2022

CHEN Hong-yan, E-mail: chen_hongyan@neau.edu.cn; CHENG Bo-han, E-mail: bhcheng@neau.edu.cn; Correspondence LI Hui, Tel/Fax: +86-451-55191516, E-mail: lihui@neau.edu.cn

[*] These authors contributed equally to this study.



doi: 10.1016/S2095-3119(21)63895-9

by targeting janus kinase 2 (*JAK2*) transcription. The results of functional rescue experiments indicated that STAT3 signaling mediated the regulation of HBP1 on chicken preadipocyte differentiation. In conclusion, HBP1 inhibits chicken preadipocyte differentiation by activating the STAT3 signaling pathway *via* directly enhancing *JAK2* expression. Our findings provided new insights for further analysis of the molecular genetic basis of chicken adipose tissue growth and development.

**Keywords:** chicken, HBP1, preadipocyte differentiation, STAT3 signaling pathway

## 1. Introduction

With the improvement of living standards, obesity has become an important factor affecting the quality of human life. Obesity is a metabolic disease caused by multiple risk factors (including heredity). The concept of a metabolic syndrome was summarized by Bonow *et al*. (2002), and is characterized by five common factors: obesity, insulin resistance/hyperinsulinemia, lipid metabolism disorder, impaired glucose tolerance or type 2 diabetes, and hypertension. As well as being the source of a metabolic syndrome, obesity can also increase the risk of cancer, damage target organs, and lead to endocrine dysfunction/infertility, as well as a series of diseases, such as reduced immune function (Pittas *et al*. 2004).

Fast-growing large broilers are the predominant birds used in broiler production worldwide. Over the past half century, the growth rate and meat yield of broilers have been significantly improved. However, along with rapid growth, excessive accumulation of body fat (especially abdominal fat) has become a prominent problem. Adipose tissue is important for energy storage in the body, and is mainly composed of adipocytes. In most species, adipose tissue begins to form before birth which depends on the increase in the number of adipocytes (Poissonnet *et al*. 1983, 1988). After birth, the expansion of adipose tissue depends on the increase in the number and size of adipocytes. The number of adipocytes is determined by adipogenesis, while the size of the adipocytes is related to degree of preadipocytes differentiation and triglyceride (TG) accumulation in lipid droplets (Rosen and MacDougald 2006). The differentiation of preadipocytes is mainly manifested by changes in cell structure and function, and the molecular mechanism involves the sequential expression of a series of adipogenesis-related genes, which are regulated by a transcriptional regulatory network and signal pathways composed of transcription factors and transcription cofactors (Farmer 2006; Rosen *et al*. 2006; Romao *et al*. 2011).

HMG-box protein 1 (HBP1) is a ubiquitous transcription factor that belongs to the high mobility (HMG) family of DNA binding proteins (Stros *et al*. 2007). A large number of studies have shown that HBP1 is an important factor regulating cell proliferation, senescence and apoptosis in various tissues and cells, and it also plays an important role in regulating cell differentiation (Tevosian *et al*. 1997; Sampson *et al*. 2001; Berasi *et al*. 2004; Pan *et al*. 2013). Studies in rats have found that HBP1 is expressed in a variety of cells including adipocytes and myoblasts, and its expression is directly related to differentiation (Lesage *et al*. 1994). In bone marrow cells, HBP1 can act as a tumor suppressor and general differentiation inducer, and can act synergistically with chemical differentiation agents to enhance lineage-specific differentiation (Lin *et al*. 2001). The increased expression of HBP1 determines the occurrence of erythroid cells, megakaryocytes, keratinocytes and cortical nerves (Borrelli *et al*. 2010; Watanabe *et al*. 2015). Three independent observations indicated the potential function of HBP1 in muscle differentiation (Maione *et al*. 1994; Corbeil *et al*. 1995; Tevosian *et al*. 1997). First, HBP1 expression increased with the differentiation of myoblasts. Second, the expression of HBP1 protein inhibited the promoter of the MYCN Proto-Oncogene (*N-myc*), while *N-myc* was downregulated in differentiated cells. Third, HBP1 expression caused cell cycle arrest, which is a necessary feature of terminal differentiation. In addition, studies have shown that HBP1 played a crucial role in chromatin remodeling events during proliferation arrest in differentiated cells by regulating the expression of specific chromatin-related proteins (such as histone H10) (Lemercier *et al*. 2000).

In our previous research, we used the blood genomes of continuous generations (4–18 generations) of fat and lean broilers at the Northeast Agricultural University, China, as experimental materials to analyze the changes in genome structure of fat and lean broilers with generation selection using the mixed pool sequencing method. The results showed that multiple genes were related to chicken abdominal fat traits, including the *HBP1* gene (Zhang *et al*. 2020). In

addition, previous studies indicated that HBP1 could inhibit the differentiation of mouse 3T3-L1 preadipocytes by activating the Wnt/β-catenin signaling pathway (Ross *et al*. 2000; Bennett *et al*. 2002). However, is HBP1 involved in regulating chicken preadipocyte differentiation? What are the signaling pathways regulated by HBP1 in preadipocyte differentiation? These issues are not known yet. Therefore, we hypothesized that HBP1 would inhibit chicken preadipocyte differentiation by activating one or some signal pathways. The aim of the current study was to clarify the biological function and mechanism of HBP1 in chicken preadipocyte differentiation.

## 2. Materials and methods

### 2.1. Experimental birds and management

The male birds used in this study were derived from the 19th generation ($G_{19}$) of the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF). NEAUHLF has been selected since 1996 using plasma very-low-density lipoprotein concentration and abdominal fat percentage (AFP) as the selection criteria: AFP (%)=AFW/BW7×100, where AFW is abdominal fat weight, and BW7 is body weight at 7 weeks of age. A previous study has described the breeding procedure in details (Guo *et al*. 2011). All birds used in this study were kept under similar environmental conditions and had free access to feed and water. Commercial corn-soybean-based diets met all nutrient requirements of broilers recommended by the National Research Council (NRC 1994) were provided to the birds.

### 2.2. Tissue collection

In total, 42 male birds (three birds for each broiler line, aged 1–7 weeks) from $G_{19}$ were sacrificed after fasting for 12 h. At the end of each week, the live weight of birds was measured before slaughtering. The average body weight of lean birds per week was (120.033±6.832), (235.133±22.682), (445.233±0.309), (578.567±10.351), (715.3±54.737), (1 185.833±107.420), and (1 805.4±34.992) g, respectively. The average body weight of fat birds per week was (129.067±7.636), (237.6±1.78), (375.5±20.792), (580.833±51.842), (799.267±46.968), (1 321.1±164.22), and (1 693.6±84.227) g, respectively. Then, the abdominal fat tissue was collected after slaughtering each week. After washing with 0.75% NaCl, all the tissues were collected, snap-frozen in liquid nitrogen, and stored at –80°C until further use.

### 2.3. Cell culture

Immortalized chicken preadipocytes (ICP2), HBP1 knockout ICP2 cells ($HBP1^{-/-}$), HBP1 overexpression CP2 cells (HBLV-HA-HBP1) and its control cells (HBLV-HA) were used in this study. These chicken cell lines were constructed previously in our laboratory (Wang *et al*. 2017; Chen *et al*. 2019). The cells were cultured in DMEM/F12 (Gibco, USA) medium containing 10% fetal bovine serum (FBS, BI, Israel).

### 2.4. Induced differentiation

The cells were seeded on a cell culture plate. After growth to 50% confluence, the medium was discarded and replaced with oleic acid (Sigma, USA) induction medium at a concentration of 300 μmol $L^{-1}$ to induce differentiation. The oleic acid induction medium was replaced every 24 h.

### 2.5. Oil red O staining and extraction

The medium in the cell culture plate was discarded and the cells were washed three times with phosphate buffered saline (PBS). After fixing for 30 min in 4% formaldehyde solution, cells were washed three times with PBS and then stained with 60% oil red O staining solution (Sigma, USA) for 15 min. After removing the staining solution, and washing three times with PBS, 60% isopropanol was added for color separation for 10–20 s. Following removal of the isopropanol and washing three times with PBS, the deposition of lipid droplets was observed and photographed under a microscope. After oil red O staining, oil red O was dissolved in 100% isopropanol and shaken on the shaking table for 15 min. Then, the OD value was measured at 510 nm with an enzyme reader. The total protein was extracted and the protein concentration was determined using a BCA Protein Quantitative Analysis Kit (Thermo Fisher Scientific, USA). The lipid droplet accumulation was presented as a ratio of $OD_{510}$ $mg^{-1}$ protein.

### 2.6. RNA preparation and reverse transcription quantitative PCR (RT-qPCR)

Total RNA was extracted using a Trizol Reagent Kit (Invitrogen, USA) following the manufacturer's protocol. First strand cDNA synthesis was performed with 1 μg of total RNA (TaKaRa, China). The qPCR was performed using the FastStart Universal SYBR Green Master Kit (Roche Molecular Systems, USA), and reaction conditions were as follows: 95°C for 10 min followed by 40 cycles

for 95°C for 1 min and 60°C for 1 min. The results were analyzed using the mean of threshold cycle (Ct) for each sample. Relative expression level of the gene was calculated using the $2^{-\Delta Ct}$ method. TATA-box binding protein (*TBP*) was used as the reference gene. The primers used for qPCR were shown in Table 1.

### 2.7. Western blot

Total proteins were extracted from the tissues or cells with RIPA lysis buffer (Beyotime, China). Then protein samples were separated by SDS/PAGE on a 12% gel and transferred to a poly (vinylidene difluoride) membrane (0.45 or 0.22 μm; Millipore, USA) and blocked with 5% Bovine serum albumin (BSA) (Sigma, USA) for 2 h. Membrane was incubated with HBP1 rabbit polyclonal antibody (Abcam, USA), PPARγ rabbit monoclonal antibody (CST, USA), A-FABP goat polyclonal antibody (Abcam, USA), janus kinase 1 (JAK1) rabbit polyclonal antibody (BOSTER, China), phospho-JAK1 rabbit polyclonal antibody (Beyotime, China), JAK2 rabbit monoclonal antibody (Beyotime, China), phospho-JAK2 rabbit polyclonal antibody (Beyotime, China), janus kinase 3 (JAK3) rabbit polyclonal antibody (Beyotime, China), phospho-JAK3 rabbit polyclonal antibody (Bioss, China), STAT3 rabbit monoclonal antibody (Beyotime, China), phospho-STAT3 rabbit polyclonal antibody (Beyotime, China) and β-actin mouse monoclonal antibody (Beyotime, China) overnight at 4°C, respectively. Then membranes were incubated with the HRP-labeled corresponding IgG at room temperature for 1 h. Finally, the target protein bands were imaged and analyzed using the Tanon-5200 Automated Chemiluminescence Analyzer (Tanon Science and Technology Co., Ltd., Shanghai, China).

**Table 1** Primers used for qPCR analysis

| Primer name[1)] | Primer sequence (5′→3′) |
|---|---|
| HBP1 | F: TGTGGGAAGTGAAGACG |
| | R: GAGAAGAGGGCAGGTTT |
| PPARγ | F: GTGCAATCAAAATGGAGCC |
| | R: CTTACAACCTTCACATGCAT |
| A-FABP | F: ATGTGCGACCAGTTTGTG |
| | R: TTGCCATCCCACTTCTG |
| JAK2 | F: TGTTCTGGATATGATGAGGGTG |
| | R: AAGGCGGACTGAAGGGTT |
| STAT3 | F: AACAGCGGTGCGGTAATG |
| | R: CAGGCAGTGAGTGGGTCT |
| TBP | F: GCGTTTTGCTGCTGTTATTATGAG |
| | R: TCCTTGCTGCCAGTCTGGAC |
| CHIP-JAK2-1 | F: AGAAGTAAGTTGCCACA |
| | R: CTTTCCTTTTGTCTTTG |
| CHIP-JAK2-2 | F: TAACAAGCAAACCTGA |
| | R: CTCCCAGCATTACAGT |
| CHIP-JAK2-3 | F: GATAATTCAGAAGGTTT |
| | R: TCTACTCATCAAGGTCA |

[1)] PPARγ, peroxisome proliferator-activated receptor gamma; A-FABP, adipocyte fatty-acid binding protein; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; TBP, TATA-box binding protein.

### 2.8. Signal pathway screening

The Cignal 45-Pathway Reporter Array (Qiagen, Germany) is suitable for use in phenotypic-related RNAi or overexpression experiments, and for studying the biological response of small molecules or compounds, as well as the mechanism of action of proteins, peptides and ligands (Lu *et al*. 2021). Therefore, we used this kit to screen the signal pathways that HBP1 regulates in the differentiation of chicken preadipocytes. The specific experimental steps were as follows: 50 μL of Opti-MEM (Invitrogen, USA) were added to each well of a 96-well plate, then the plate was shaken gently and incubated at room temperature for 5 min. Next, 60 μL of Attractene (Qiagen, Germany) was added to 5 000 μL of Opti-MEM, and incubated for 5 min at room temperature. Then, 50 μL of the reagent from step 2 was added to each well of the 96-well plate, which was gently shaken and then incubated at room temperature for 20 min. The HBLV-HA and HBLV-HA-HBP1 cells were taken out of the incubator, the culture medium was discarded, and the cells were washed three times with PBS. Then, the cells were digested with 0.25% trypsin for 1–2 min. The digestion was stopped with Opti-MEM containing 5% FBS, followed by centrifugation at 800×g for 5 min. After discarding the supernatant, the cells were resuspended in an appropriate amount of Opti-MEM containing 10% FBS and counted. Then, 50 μL of cell suspension (about $10^4$ cells) was added to each well and the 96-well plate was gently shaken to ensure the cells were evenly inoculated, and the plate was placed in an incubator for cell culture. After growth to 50% confluence, the original medium was discarded and 150 μL of oleic acid medium was added to each well, replacing the oleic acid induction medium every 24 h and continuing culturing until 72 h. At 72 h post-induction, the cells were collected and the activity of the dual luciferase reporter gene was detected.

### 2.9. Dual-luciferase reporter assays

Assays were performed according to the instructions of the dual luciferase reporter assay system (Promega, USA). The steps were as follows: the culture medium was removed from the culture plate and wells were washed three times with PBS. Then, 20 μL of 1×PLB was added to each well and the cell culture plate was shaken horizontally at room temperature for 15 min.

The cell lysate was then transferred to a new 1.5 mL centrifuge tube, centrifuged at 14 000×g for 5 min, and the supernatant was collected. Next, 50 μL of LARII that had been equilibrated to room temperature was added to a new 1.5-mL centrifuge tube, and then, 10 μL of PLB lysate supernatant was added to the 1.5-mL centrifuge tube to detect the activity of firefly luciferase (Fluc). Following this step, 50 μL of Stop&Glo was added to detect the activity of renilla luciferase (Rluc). The relative luciferase activity was presented as a ratio of Fluc/Rluc.

### 2.10. Application of the signal pathway chemical inhibitor

ICP2 cells were seeded into both 96-well and 12-well plates. After growth to 50% confluence, the medium was discarded and replaced with oleic acid induction medium containing different concentrations (0, 50, 100, 200, and 400 nmol $L^{-1}$) of STAT3 inhibitor Stattic (MCE, USA). The medium was changed every 24 h until 72 h. A CCK-8 Kit (Dojindo, Japan) was used to detect the effect of adding Stattic on cell viability. Oil red O staining was used to detect the influence of the inhibitor on lipid droplet accumulation, and Western blot was used to analyze the effect of the inhibitor on the phosphorylation level of STAT3.

### 2.11. Cell transfection

HBLV-HA-HBP1 and HBLV-HA cells were plated on a 12-well cell culture plate. After growth to 60% confluence, STAT3-siRNA or the negative control (NC)-siRNA were transfected into the cells by using Liposome 3000 reagent (Invitrogen, USA). The siRNAs were synthesized commercially (Gene Pharma, China). The siRNA sequences were shown in Table 2.

### 2.12. Chromatin immunoprecipitation (ChIP)

HBLV-HA-HBP1 cells were seeded at $4\times10^6$ cells per 15-cm dish. After growth to 50% confluence, the original medium was discarded and replaced with 20 mL of oleic acid medium. The oleic acid induction medium was changed every 24 h until 72 h. The cells were crosslinked with 37% formaldehyde for 10 min. Glycine was then added for 5 min to a final concentration of 0.125 mol $L^{-1}$, and the cells were scrapped and collected. Follow-up ChIP experiments were carried out in accordance with the instructions for the Simple ChIP® Plus Enzymatic Chromatin IP Kit (Agarose Beads, CST, USA). After cell cross-linking and preparation, chromatin concentration and enzyme digestion analysis, the chromatins were divided into three sections which used as input and used for immunoprecipitation by anti-HA tag antibody (Abcam, USA) and IgG antibody in the ChIP Kit, respectively. After chromatin immunoprecipitation, the chromosomes were eluted from the antibody/protein G beads and de-crosslinked. Then, DNA was purified through a spin column and the purified DNA was used for qPCR analysis. ChIP-qPCR primer sequences were shown in Table 1. The qPCR system comprised a 20-μL reaction volume and the cycle conditions were: 95°C for 10 min, then 95°C for 20 s and 60°C for 30 s for 40 cycles. The results of ChIP-qPCR were presented as the fold enrichment, which was calculated according to the following formula:

$\Delta Ct_{normalized\ ChIP}=Ct_{HA}-(Ct_{Input}-\log_2 Input\ dilution\ factor)$, $\Delta Ct_{normalized\ NIS}=Ct_{IgG}-(Ct_{Input}-\log_2 Input\ dilution\ factor)$; $\Delta\Delta Ct_{ChIP/NIS}=\Delta Ct_{normalized\ ChIP}-\Delta Ct_{normalized\ NIS}$; Fold enrichment=$2^{-\Delta\Delta Ct_{ChIP/NIS}}$. Input dilution factor=1/Fraction of the input chromatin saved. Input accounts for 5% of the total chromatin in this experiment, so the input dilution factor was 20 (Patrik 2018; Cui *et al*. 2021).

**Table 2** The siRNA sequence used in this study

| Name | Sequence (5′→3′) |
|---|---|
| STAT3-Gallus-865 | Sense: GCUGUCAGCCAUGGAGUAUTT |
| | Antisense: AUACUCCAUGGCUGACAGCTT |
| STAT3-Gallus-931 | Sense: GCAACAAAUUGCCUGCAUUTT |
| | Antisense: AAUGCAGGCAAUUUGUUGCTT |
| STAT3-Gallus-1856 | Sense: GCAGGCAAAGGCUUCUCUUTT |
| | Antisense: AAGAGAAGCCUUUGCCUGCTT |
| NC-siRNA | Sense: UUCUCCGAACGUGUCACGUTT |
| | Antisense: ACGUGACACGUUCGGAGAATT |

### 2.13. Statistical analysis

The signal pathway screening experiment was repeated twice, and the remaining experiments were repeated three times. Data are shown as the mean±SD. The expression level of HBP1 during differentiation of ICP2 cells was analyzed using one-way ANOVA, and the differences in expression level of HBP1 among various time points were analyzed by the Duncan method. The trend of *HBP1* mRNA expression level during differentiation of ICP2 cells was analyzed by an orthogonal comparison including linear and quadratic components (Richard 2008). The mRNA expression level of *STAT3* after STAT3-siRNA transfection was analyzed using one-way ANOVA, and the differences in mRNA expression level of *STAT3* between control group and treatment group were analyzed by the Dunnett method. The effect of inhibition of STAT3 activity or knockdown of STAT3 on HBP1 mediated inhibition of preadipocyte differentiation was analyzed using two-way ANOVA (the statistical model included the supplement of

Stattic, overexpression of HBP1 and their interaction; the transfection of STAT3 siRNA, overexpression of HBP1 and their interaction), and the differences in lipid droplet deposition and expression level of PPARγ and A-FABP between the groups were analyzed by the Duncan method. The other data were analyzed using Student's *t*-test. For experiments using tissues, each individual was the experimental unit. For experiments using cells, each well was the experimental unit. Differences were considered statistically significant at $P<0.05$. All analyses were performed by SPSS 16.0 Satistical Software.

## 3. Results

### 3.1. The expression pattern of chicken HBP1 in abdominal adipose tissue and during preadipocyte differentiation

The RT-qPCR results showed that the mRNA expression levels of *HBP1* in the abdominal adipose tissue of fat and lean broilers were significantly different at 1, 2, 5, 6, and 7 weeks of age. At 1, 5, 6, and 7 weeks of age, the lean broilers showed higher ($P<0.05$) mRNA expression levels of *HBP1* than those of the fat broilers, and at 2 weeks of age, the fat broilers showed higher ($P<0.05$) mRNA expression levels of *HBP1* than those of the lean broilers (Fig. 1-A). Western blot analysis showed that the protein expression levels of HBP1 in the abdominal adipose tissue of fat and lean broilers were significantly different at 1, 2, 3, 5, 6, and 7 weeks of age. At 1, 5, 6, and 7 weeks of age, lean broilers showed higher ($P<0.05$) protein expression levels of HBP1 than those of the fat broilers, and at 2 and 3 weeks of age, fat broilers showed higher ($P<0.05$) protein expression levels of HBP1 than those of the lean broilers (Fig. 1-B and C). To understand whether the HBP1 was involved in the differentiation of chicken preadipocytes, we investigated the expression pattern of HBP1 during the differentiation of ICP2 cells. The results of Oil red O staining showed that as differentiation progressed, the accumulation of lipid droplets increased (Fig. 1-D). The result of Duncan's multiple comparison showed that the mRNA expression level of *HBP1* increased firstly and then decreased ($P<0.05$) during the differentiation of ICP2 cells (Fig. 1-E). At the same time, an orthogonal comparison was utilized to analyze the trend of *HBP*1 mRNA expression level during the differentiation of ICP2 cells. The result showed that the quadratic polynomial fitted best ($P=0.0002$) and displayed the changing trend of *HBP1* mRNA expression level (Fig. 1-E). Although the protein expression level of HBP1 did not change significantly ($P>0.05$) during the differentiation of ICP2 cells, the trend was very similar to that of *HBP1* mRNA (Fig. 1-F and G).

### 3.2. HBP1 is a negative regulator of chicken preadipocyte differentiation

The constructed HBP1 overexpression preadipocytes, HBP1 knockout preadipocytes and their control cells were used to study the effects of HBP1 on the differentiation of chicken preadipocytes. The results showed that compared with the control group, overexpression of HBP1 significantly inhibited ($P<0.01$ or $P<0.05$) lipid droplet deposition (Fig. 2-A and B) and the expression of PPARγ and A-FABP (Fig. 2-C–G). In contrast, compared with the control group, knockout of HBP1 significantly promoted ($P<0.05$) lipid droplet deposition (Fig. 2-H and I) and the expression of PPARγ and A-FABP (Fig. 2-J–N). These results indicated that HBP1 negatively regulated the differentiation of chicken preadipocytes.

### 3.3. HBP1 activates the STAT3 signal by upregulating *JAK2* expression

To explore the molecular mechanism of HBP1 inhibition of the differentiation of chicken preadipocytes, we used the dual luciferase reporter gene system to analyze the signal pathway employed by HBP1 to regulate the differentiation of chicken preadipocytes. The results showed that the STAT3 signaling pathway was significantly activated ($P<0.05$) when HBP1 was overexpressed (Fig. 3-A). Moreover, Western blot analysis also showed that overexpression of HBP1 significantly promoted ($P<0.05$) the phosphorylation of STAT3 (Fig. 3-B and C), whereas knockout of HBP1 significantly inhibited ($P<0.05$) the phosphorylation of STAT3 (Fig. 3-D and E). Studies in mammals have shown that JAK2-STAT3 signaling played an important role in adipogenesis (Wu *et al*. 2019). Therefore, we investigated whether HBP1 enhanced the phosphorylation level of JAK2 in the differentiation of chicken preadipocytes. Western blot analysis showed that overexpression of HBP1 significantly enhanced ($P<0.001$) the phosphorylation of JAK2 (Fig. 3-B and C), whereas knockout of HBP1 significantly inhibited ($P<0.001$) the phosphorylation level of JAK2 (Fig. 3-D and E). It is worth noting that overexpression of HBP1 enhanced ($P=0.076$) the protein expression of JAK2 (Fig. 3-B and C). Because of HBP1 is a transcription factor, we wondered whether HBP1 could activate *JAK2* transcription. The RT-qPCR results showed that overexpression of HBP1 significantly upregulated ($P<0.05$) the mRNA expression of *JAK2* (Fig. 3-F). Next, we used JASPAR (http://jaspar.genereg.net/) to predict the HBP1 binding sites within the promoter region (–2 000 to +1 bp from the transcriptional start site)

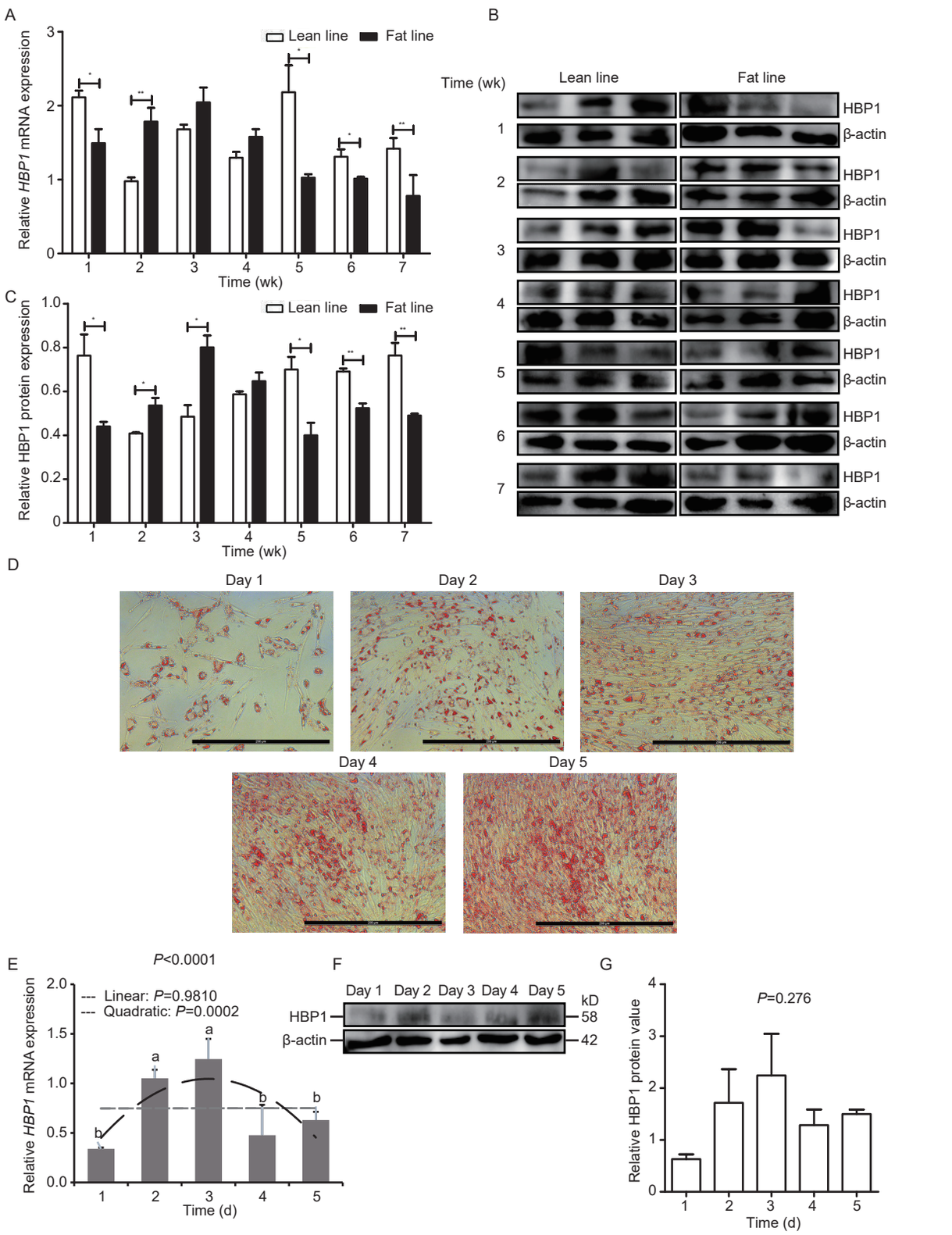


**Fig. 1** The expression pattern of chicken HMG-box protein 1 (HBP1) in abdominal adipose tissue and preadipocyte differentiation. A, *HBP1* mRNA expression in the abdominal adipose tissue of fat and lean broilers at 1–7 weeks of age. B and C, protein expression of HBP1 in the abdominal adipose tissue of fat and lean broilers at 1–7 weeks of age. D, oil red O staining over 1–5 days of Immortalized chicken preadipocytes (ICP2) differentiation. Scale bar: 200 μm. E, *HBP1* mRNA expression during ICP2 differentiation over 1–5 days. F and G, HBP1 protein expression during ICP2 differentiation over 1–5 days. ICP2 cells were photographed under a light microscope. Data are presented as the mean±SD ($n$=3). Means without a common letter differ ($P$<0.05). The $P$-values of orthogonal comparison were marked on the graph. *, $P$<0.05; **, $P$<0.01.

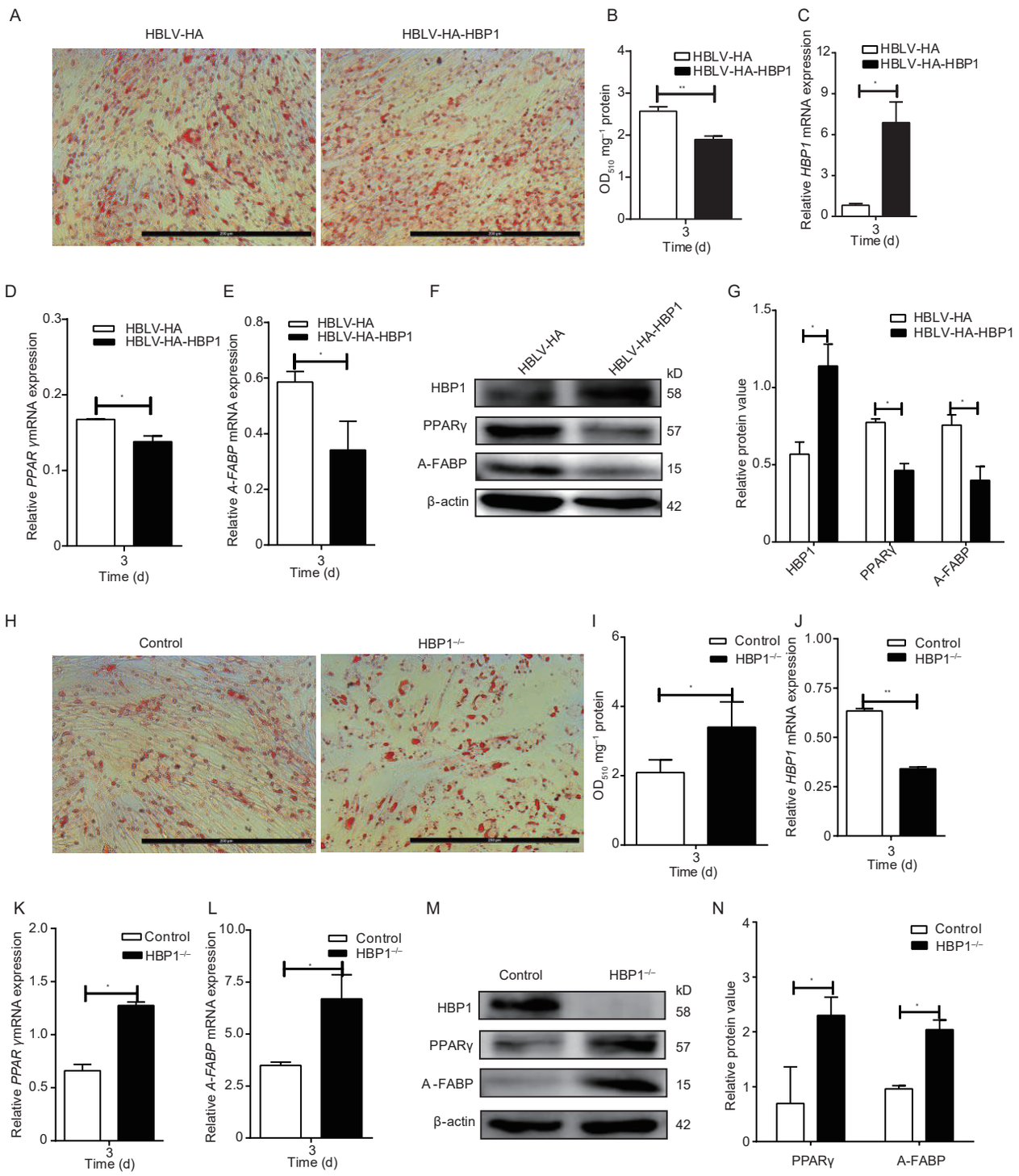


**Fig. 2** The effect of HBP1 on the differentiation of chicken preadipocytes. A and B, oil red O staining and colorimetric extraction of HBLV-HA and HBLV-HA-HBP1 after 3 days of differentiation. C–E, the mRNA expression of *HBP1*, *PPARγ* and *A-FABP* after 3 days of differentiation of HBLV-HA and HBLV-HA-HBP1. F and G, protein expression of HBP1, PPARγ and A-FABP after 3 days of differentiation of HBLV-HA and HBLV-HA-HBP1. H and I, oil red O staining and colorimetric extraction of the control and HBP1$^{-/-}$ after 3 days of differentiation. J–L, the mRNA expression of *HBP1*, *PPARγ* and *A-FABP* after 3 days of differentiation of the control and HBP1$^{-/-}$. M and N, protein expression of PPARγ and A-FABP after 3 days of differentiation of the control and HBP1$^{-/-}$. ICP2 cells were photographed under a light microscope. HBLV-HA-HBP1, ICP2 cells infected by lentivirus over-expressing HBP1; HBLV-HA, ICP2 cells infected by control lentivirus; HBP1$^{-/-}$, HBP1 knockout ICP2 cells; control, wildtype ICP2 cells; PPARγ, peroxisome proliferator-activated receptor gamma; A-FABP, adipocyte fatty-acid binding protein. Scale bar: 200 μm. Data are presented as the mean±SD ($n$=3). $^{*}$, $P$<0.05; $^{**}$, $P$<0.01.

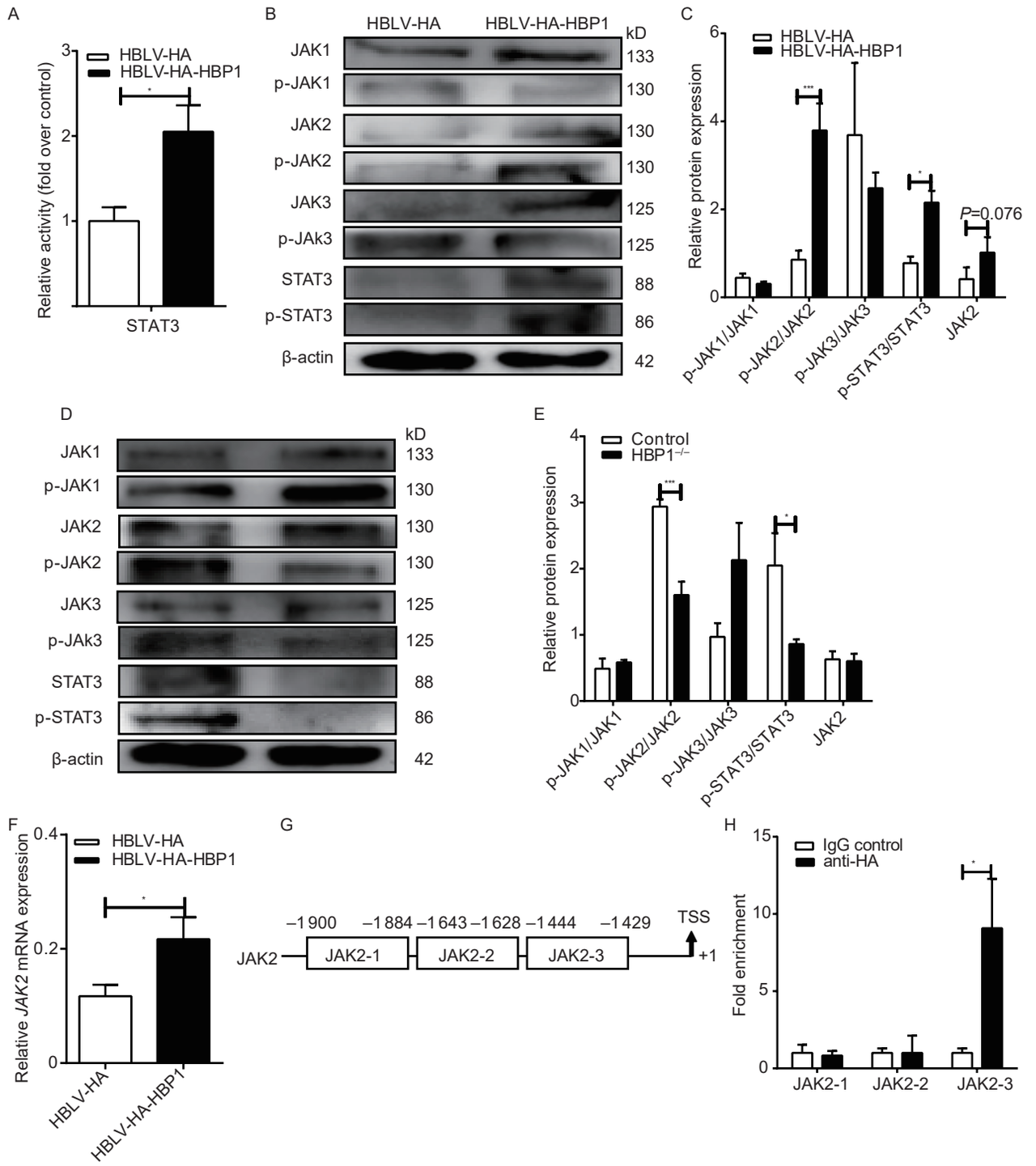


**Fig. 3** HBP1 activates the JAK2-STAT3 signaling pathway by targeting and promoting the transcription of JAK2. A, the dual luciferase reporter gene system was used to screen the signal pathway of HBP1 in regulating the differentiation of chicken preadipocytes. B and C, Western blot detection of protein expression when HBP1 was overexpressed. D and E, Western blot detection of protein expression when HBP1 was knocked out. F, the effect of HBP1 overexpression on *JAK2* mRNA expression was detected by RT-qPCR. G, a schematic model depicting the potential binding site of HBP1 in the *JAK2* promoter region. H, ChIP-qPCR. HBLV-HA, ICP2 cells infected by control lentivirus; HBLV-HA-HBP1, ICP2 cells infected by lentivirus over-expressing HBP1; p-JAK1, p-JAK2, p-JAK3 and p-STAT3, phosphorylated janus kinase 1 (JAK1), phosphorylated JAK2, phosphorylated JAK3 and phosphorylated signal transducer and activator of transcription 3 (STAT3), respectively; control, wild type ICP2 cells; HBP1$^{-/-}$, HBP1 knockout ICP2 cells; IgG control, chromatin immunocoprecipitation with IgG; anti-HA, chromatin immunocoprecipitation with HA-tag antibody; JAK2-1, JAK2-2 and JAK2-3, the potential HBP1 binding site in the promoter region of *JAK2* gene; TSS, transcriptional start site. Data are presented as the mean±SD ($n$=3). $^{*}$, $P$<0.05; $^{***}$, $P$<0.001.

of *JAK2* and found that there were three potential HBP1 binding sites (designated JAK2-1, JAK2-2, and JAK2-3; Fig. 3-G) in the promoter region of chicken *JAK2* gene. The ChIP-qPCR results showed that HBP1 bound ($P$<0.05) to JAK2-3 site (–1 429 to –1 444 bp) (Fig. 3-H). These results suggested that HBP1 activated the STAT3 signal by promoting the transcription of *JAK2*.

### 3.4. HBP1 inhibits the differentiation of chicken preadipocytes by activating the STAT3 signal

Next, we investigated whether the STAT3 signal mediated the regulation of HBP1 on chicken preadipocyte differentiation. Stattic, a STAT3 inhibitor, was added to ICP2 cells to inhibit the activity of STAT3. We determined that the optimal concentration of Stattic was 400 nmol $L^{-1}$ as this concentration significantly inhibited ($P$<0.05) the phosphorylation of STAT3 and promoted ($P$<0.05) lipid droplet deposition (Appendix A). In addition, we suppressed the expression of STAT3 by siRNA mediate knockdown. STAT3-siRNA 3 was identified as an effective interference fragment, because STAT3-siRNA3 significantly inhibited ($P$<0.05) the expression of STAT3 and promoted ($P$<0.05) lipid droplet accumulation (Appendix B). Finally, we used the inhibitor and siRNA of STAT3 to carry out functional rescue experiments. The results showed that the inhibition of STAT3 activity attenuated the inhibitory effect of overexpression of HBP1 on the differentiation of chicken preadipocytes. After the HBP1 overexpression cells were treated with Stattic, lipid droplet deposition and the expression levels of PPARγ and A-FABP increased, and the level of phosphorylated STAT3 decreased ($P$<0.05) significantly (Fig. 4-A–D). Similarly, the inhibition of STAT3 expression attenuated the inhibitory effects of overexpression of HBP1 on the differentiation of chicken preadipocytes. After the HBP1 overexpression cells were transfected by STAT3-siRNA, lipid droplet deposition increased, the expression level of PPARγ also increased ($P$<0.05), and the expression level of STAT3 was significantly reduced ($P$<0.05) (Fig. 5-A–D). These results suggested that HBP1 inhibited the differentiation of chicken preadipocytes by activating the STAT3 signal.

## 4. Discussion

HBP1 is a ubiquitous transcription factor that belongs to HMG family of DNA binding proteins (Stros *et al*.

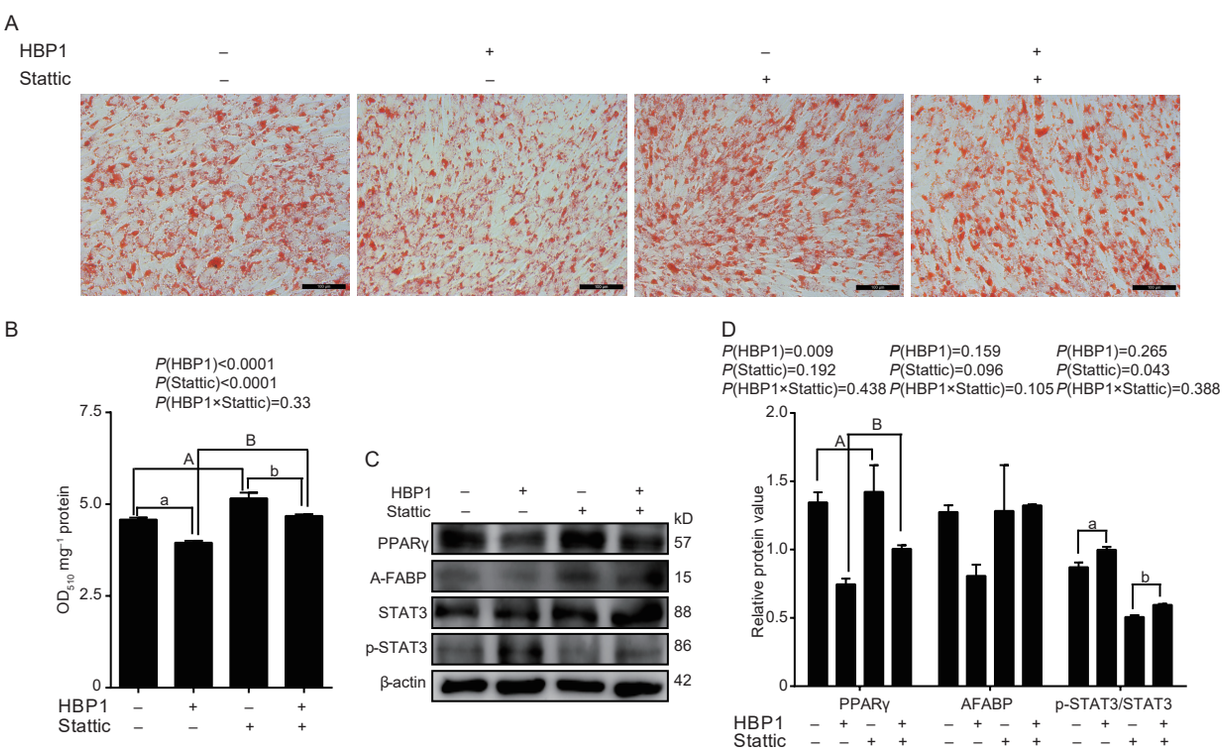


**Fig. 4** STAT3 inactivation reverses the inhibitory effect of HBP1 on chicken preadipocyte differentiation. A and B, oil red O staining and oil red O extraction colorimetric analysis. Scale bar: 100 μm. C and D, Western blot detection of related protein changes. p-STAT3, phosphorylated STAT3. Data are presented as the mean±SD ($n$=3). Means without a common letter differ ($P$<0.05).

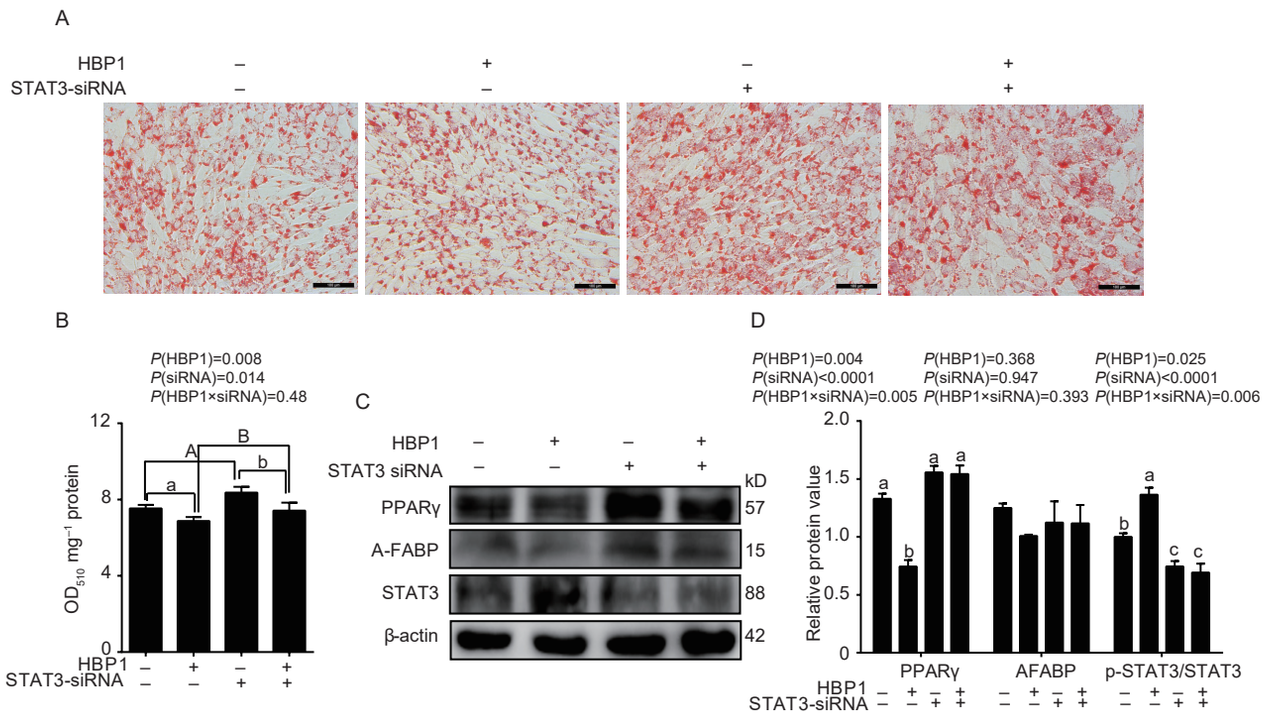


**Fig. 5** STAT3 knockdown reverses the inhibitory effect of HBP1 on chicken preadipocyte differentiation. A and B, oil red O staining and oil red O extraction colorimetric analysis. Scale bar: 100 μm. C and D, Western blot detection of related protein changes. Data are presented as the mean±SD ($n$=3). Means without a common letter differ ($P$<0.05).

2007). Several researches have showed that HBP1 could inhibit the differentiation of mouse 3T3-L1 preadipocytes by activating the Wnt/β-catenin signaling pathway (Ross *et al*. 2000; Bennett *et al*. 2002). However, the function and regulatory mechanisms of HBP1 in chicken preadipocyte differentiation are not clear. In the present study, our hypothesis that HBP1 would inhibit chicken preadipocyte differentiation by activating one or some signal pathways has been supported by the results of the present study. In this study, first, we found that overexpression/knockout of HBP1 in chicken preadipocytes could inhibit/promote lipid droplet deposition and the expression of adipogenesis-related genes. Second, the results of luciferase reporter assay and Western blot showed that STAT3 signaling pathway was activated by overexpression of HBP1. Finally, the results of functional rescue experiments indicated that STAT3 signaling mediated the regulation of HBP1 on chicken preadipocyte differentiation. These results suggested that HBP1 inhibits chicken preadipocyte differentiation by activating JAK2-STAT3 signaling pathway (Fig. 6). This study provided better understanding of the molecular genetic basis of chicken adipose tissue growth and development, and provided new insight into strategies for controlling the excessive accumulation of body fat in broilers.

In the current study, we found that the expression of HBP1 existed a significant difference in abdominal fat between the fat and lean broilers. We also found that HBP1 expression increased firstly and then decreased during the differentiation of ICP2 cells (Fig. 1). These results suggested that HBP1 may play an important regulatory role in chicken preadipocytes differentiation, thereby affecting fat deposition. Subsequently, we found that overexpression of HBP1 could significantly inhibit lipid droplet deposition and the expression of PPARγ and A-FABP, while knockout of HBP1 had the opposite effect (Fig. 2). Our results were consistent with the previous study that HBP1 inhibits rat preadipocyte differentiation at the early stage (Chan *et al*. 2018). PPARγ is an important transcription factor regulating adipogenesis, which has been confirmed by the experiments *in vivo* and *in vitro* in mice (El-Jack *et al*. 1999). Our previous studies also demonstrated that PPARγ palys a key role in chicken preadipocyte differentiation (Liu *et al*. 2010; Sun *et al*. 2014; Wang *et al*. 2018). A-FABP plays an important role in lipid metabolism in mammals and chickens (Schaap *et al*. 2002; Chmurzynska *et al*. 2006). Due to the expression level of A-FABP

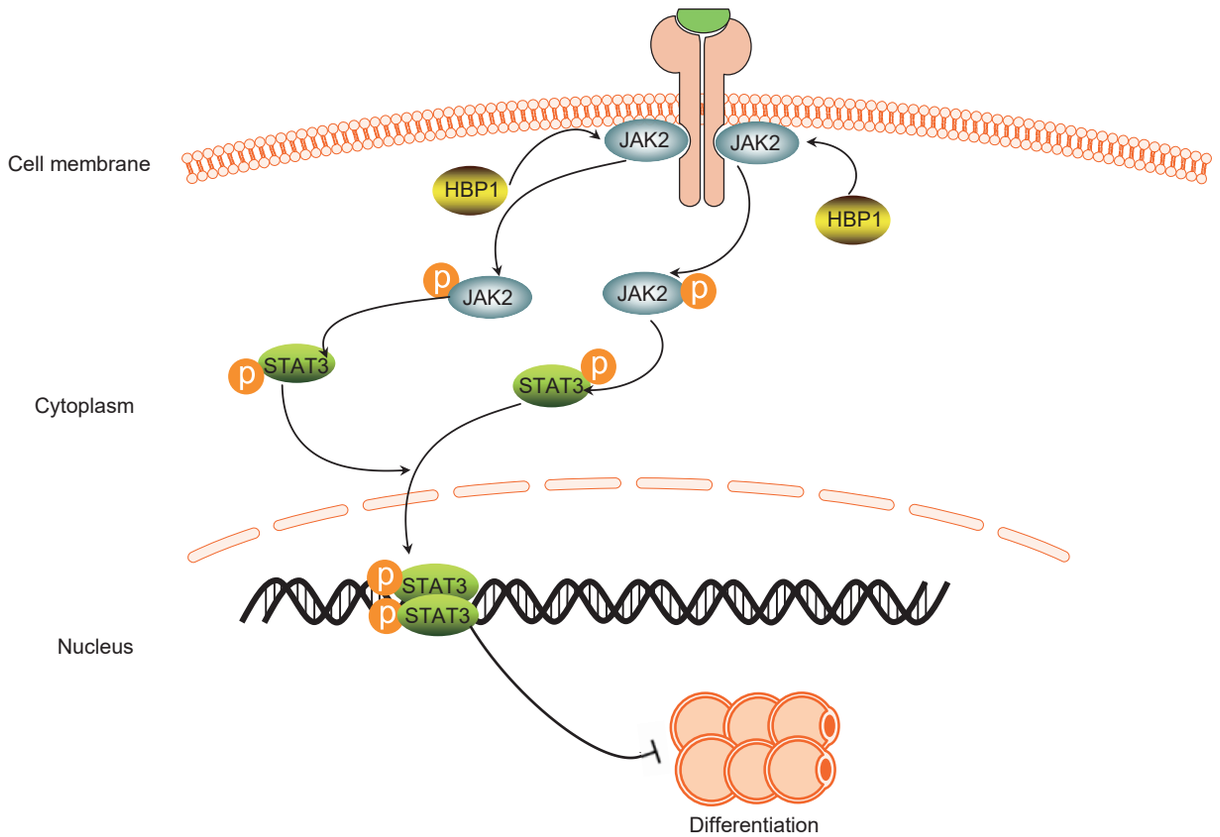


**Fig. 6** A schematic model depicting HBP1 regulating the differentiation of chicken preadipocytes.

continues to increase during preadipocyte differentiation, it is considered to be a late marker of adipogenesis (MacDougald and Lane 1995). So far, there are no reports about the regulation of PPARγ and A-FABP by HBP1. In this study, we used JASPAR online tool to predict the HBP1 binding sites within the promoter region of *PPARγ* and *A-FABP*, and found that there were two and four potential HBP1 binding sites in *PPARγ* and *A-FABP* promoter (–2 000 to +1 bp from the transcriptional start site), respectively (data not shown). Therefore, we speculated that HBP1 inhibits the transcription of *PPARγ* and *A-FABP* by binding to their promoters, which in turn inhibits adipogenesis. Taken together, our findings indicated that HBP1 is a negative regulator in chicken preadipocyte differentiation.

To further investigate the molecular mechanism of HBP1 inhibition on chicken preadipocytes differentiation, we employed a Cignal Finder Signal Transduction 45 pathway reporter array (Fig. 3-A) and found that overexpression of HBP1 could activate STAT3 signal pathway, which was further confirmed by Western blot analysis (Fig. 3-B–E). The Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway is an intracellular signal transduction pathway that is involved in many important biological processes such as cell proliferation, differentiation, apoptosis and immune regulation (Morris *et al*. 2018). Studies have shown that HBP1 can bind to JAK protein and cause the activation of cytoplasmic JAK tyrosine kinase, which causes phosphorylation at Tyr705 and Ser757 of STAT3, thereby activating the JAK-STAT signaling pathway (Nakagawa *et al*. 2006; Rauvala and Rouhiainen 2007). Studies have also shown that STAT3 plays a role in cell signal transduction mainly through the JAK2-STAT3 pathway in mammals (Levy and Darnell 2002). However, whether HBP1 activates the STAT3 signal through JAK2 is unknown in birds. In the present study, we found that overexpression of HBP1 increased the phosphorylation of JAK2 rather than JAK1 and JAK3 (Fig. 3-B–E), but the mechanism how HBP1 enhanced the phosphorylation of JAK2 remains to be determined. Considering that HBP1 is a transcription factor (Stros *et al*. 2007), and overexpression of HBP1 promotes the protein expression of JAK2 (Fig. 3-B and C), we wondered whether HBP1 could activate *JAK2* transcription. Consistent with our expectation, we found that HBP1 could bind to the *JAK2* promoter and enhance its mRNA expression (Fig. 3-F–H).

Studies have shown that the activation of STAT3 signal was related to cell differentiation. Previous studies have shown that STAT3 could promote the differentiation of astrocytes and B cells (Gires *et al*. 1999; Ohtani *et al*. 2000). Whereas, in other cell types, STAT3 has been found to inhibit cell differentiation, such as PC12 cells and embryonic stem (ES) cells (Ihara *et al*. 1997; Ernst *et al*. 1999). However, related research in poultry has not been reported. Stattic is a potent STAT3 inhibitor that can inhibit the phosphorylation of STAT3 (at sites Y705 and S727) (Lin *et al*. 2016; Wang *et al*. 2020). When the phosphorylation of STAT3 is inhibited by Stattic, STAT3 is unable to form phosphate dimers, and thus cannot be transferred from the cytoplasm to the nucleus to regulate gene expression, which ultimately results in the inhibition of activation of the JAK2-STAT3 signaling pathway (Nakagawa *et al*. 2006; Rauvala *et al*. 2007). This study found that Stattic not only significantly inhibited the phosphorylation of STAT3, but also promoted the lipid droplet accumulation in ICP2 cells (Appendix A), suggesting activation of STAT3 signaling pathway could inhibit the differentiation of chicken preadipocytes. A previous study reported that FTO alpha-ketoglutarate dependent dioxygenase (FTO) promoted mouse 3T3-L1 preadipocytes differentiation by activating STAT3 signals (Wu *et al*. 2019). Our results were not consistent with the research reported by Wu *et al*. (2019). This discrepancy may be due to the differences in lipogenesis patterns in mammals and birds (Gondret *et al*. 2001) or cell models and induced differentiation methods. In addition, we found that the inhibition of STAT3 activity reversed the inhibitory effect of overexpression of HBP1 on the differentiation of chicken preadipocytes (Figs. 4 and 5), indicating that STAT3 mediated the regulation of HBP1 on the differentiation of chicken preadipocytes.

## 5. Conclusion

In summary, our results demonstrated that HBP1 inhibits chicken preadipocyte differentiation by activating JAK2-STAT3 signaling pathway. However, the mechanism how STAT3 regulated the differentiation of chicken preadipocytes remains to be determined in the future.

## Acknowledgements

This research was supported by the China Agriculture Research System of MOF and MARA of China (CARS-41). We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

## Ethical approval

All animal work was conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of the People's Republic of China (2006-398) and approved by the Laboratory Animal Management Committee of the Northeast Agricultural University High and Low Fat (NEAUHLF), China.

**Appendices** associated with this paper are available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

## References

Bennett C N, Ross S E, Longo K A, Bajnok L, Hemati N, Johnson K W, Harrison S D, MacDougald O A. 2002. Regulation of Wnt signaling during adipogenesis. *Journal of Biological Chemistry*, **277**, 30998–31004.

Berasi S P, Xiu M, Yee A S, Paulson K E. 2004. HBP1 repression of the *p47phox* gene: Cell cycle regulation *via* the NADPH oxidase. *Molecular Cell Biology*, **24**, 3011–3024.

Bonow R O, Mitch W E, Nesto R W, O'Gara P T, Becker R C, Clark L T, Hunt S, Jialal I, Lipshultz S E, Loh E. 2002. Prevention conference VI: Diabetes and cardiovascular disease: Writing group V: Management of cardiovascular-renal complications. *Circulation*, **105**, e159–e164.

Borrelli S, Candi E, Hu B, Dolfini D, Ravo M, Grober O M, Weisz A, Dotto G P, Melino G, Vigano M A, Mantovani R. 2010. The p63 target HBP1 is required for skin differentiation and stratification. *Cell Death and Differentiation*, **17**, 1896–1907.

Chan C Y, Yu P, Chang F T, Chen Z H, Lee M F, Huang C Y. 2018. Transcription factor HMG box-containing protein 1 (HBP1) modulates mitotic clonal expansion (MCE) during adipocyte differentiation. *Journal of Cellular Physiology*, **233**, 4205–4215.

Chen H Y, Liu C, Liu Y M, Li H, Cheng B H. 2019. Transcription factor HBP1: A regulator of senescence and apoptosis of preadipocytes. *Biochemical and Biophysical Research Communications*, **517**, 216–220.

Chmurzynska A. 2006. The multigene family of fatty acid-binding proteins (FABPs): Function, structure and polymorphism. *Journal of Applied Genetics*, **47**, 39–48.

Corbeil H B, Whyte P, Branton P E. 1995. Characterization of transcription factor E2F complexes during muscle and neuronal differentiation. *Oncogene*, **11**, 909–920.

Cui T T, Huang J X, Sun Y N, Ning B L, Mu F, You X, Guo Y Q, Li H, Wang N. 2021. KLF2 inhibits chicken preadipocyte differentiation at least in part via directly repressing PPARγ

transcript variant 1 expression. *Frontiers in Cell and Developmental Biology*, **9**, 627102.

Ernst M, Novak U, Nicholson S E, Layton J E, Dunn A R. 1999. The carboxyl-terminal domains of gp130-related cytokine receptors are necessary for suppressing embryonic stem cell differentiation. *Journal of Biological Chemistry*, **274**, 9729–9737.

Farmer S R. 2006. Transcriptional control of adipocyte formation. *Cell Metabolism*, **4**, 263–273.

Gires O, Kohlhuber F, Kilger E, Baumann M, Kieser A, Kaiser C, Zeidler R, Scheffer B, Ueffing M, Hammerschmidt W. 1999. Latent membrane protein 1 of Epstein-Barr virus interacts with JAK3 and activates STAT proteins. *EMBO Journal*, **18**, 3064–3073.

Gondret F, Ferre P, Dugail I. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. *Journal of Lipid Research*, **42**, 106–113.

Guo L, Sun B, Shang Z, Leng L, Y Wang Y, Wang N, Li H. 2011. Comparison of adipose tissue cellularity in chicken lines divergently selected for fatness. *Poultry Science*, **90**, 2024–2034.

Ihara S, Nakajima K, Fukada T, Hibi M, Nagata S, Hirano T, Fukui Y. 1997. Dual control of neurite outgrowth by STAT3 and MAP kinase in PC12 cells stimulated with interleukin-6. *EMBO Journal*, **16**, 5345–5352.

El-Jack A K, Hamm J K, Pilch P F, Farmer S R. 1999. Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARγ and C/EBPα. *Journal of Biological Chemistry*, **274**, 7946–7951.

Lemercier C, Duncliffe K, Boibessot I, Zhang H, Verdel A, Angelov D, Khochbin S. 2000. Involvement of retinoblastoma protein and HBP1 in histone H1(0) gene expression. *Molecular and Cellular Biology*, **20**, 6627–6637.

Lesage F, Hugnot J P, Amri E Z, Grimaldi P, Barhanin J, Lazdunski M. 1994. Expression cloning in $K^+$ transport defective yeast and distribution of HBP1, a new putative HMG transcriptional regulator. *Nucleic Acids Research*, **22**, 3685–3688.

Levy D E, Darnell J J. 2002. Stats: Transcriptional control and biological impact. *Nature Reviews Molecular Cell Biology*, **3**, 651–662.

Lin K M, Zhao W G, Bhatnagar J, Zhao W D, Lu J P, Simko S, Schueneman A, Austin G E. 2001. Cloning and expression of human HBP1, a high mobility group protein that enhances myeloperoxidase (MPO) promoter activity. *Leukemia*, **15**, 601–612.

Lin L, Jou D, Wang Y N, Ma H Y, Liu T S, Fuchs J, Li P K, Lu J G, Li C L, Lin J Y. 2016. STAT3 as a potential therapeutic target in $ALDH^+$ and $CD44^+/CD24^+$ stem cell-like pancreatic cancer cells. *International Journal of Surgical Oncology*, **49**, 2265–2274.

Liu S, Wang Y X, Wang L, Wang N, Li Y M, Li H. 2010. Transdifferentiation of fibroblasts into adipocyte-like cells by chicken adipogenic transcription factors. *Comparative Biochemistry and Physiology* (A: Molecular & Integrative Physiology), **156**, 502–508.

Lu W Q, Ni Z, Jiang S Q, Tong M F, Zhang J, Zhao J, Feng C C, Jia Q Y, Wang J Y, Yao T T, Ning H B, Shi Y Q. 2021. Resveratrol inhibits bile acid-induced gastric intestinal metaplasia *via* the PI3K/AKT/p-FoxO4 signalling pathway. *Phytotherapy Research*, **35**, 1495–1507.

MacDougald O A, Lane M D. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. *Annual Review of Biochemistry*, **64**, 345–373.

Maione R, Fimia G M, Holman P, Schaffhausen B, Amati P. 1994. Retinoblastoma antioncogene is involved in the inhibition of myogenesis by polyomavirus large T antigen. *Cell Growth and Differentiation*, **5**, 231–237.

Morris R, Kershaw N J, Babon J J. 2018. The molecular details of cytokine signaling *via* the JAK/STAT pathway. *Protein Science*, **27**, 1984–2009.

Nakagawa T, Hu H, Zharikov S, Tuttle K R, Short R A, Glushakova O, Ouyang X, Feig D I, Block E R, Herrera-Acosta J, Patel J M, Johnson R J. 2006. A causal role for uric acid in fructose-induced metabolic syndrome. *American Journal of Physiology* (Renal Physiology), **290**, F625–F631.

NRC (National Research Council). 1994. *Nutrient Requirements of Poultry*. 9th ed. National Academies Press, Washington, D.C.

Ohtani T, Ishihara K, Atsumi T, Nishida K, Kaneko Y, Miyata T, Itoh S, Narimatsu M, Maeda H, Fukada T, Itoh M, Okano H, Hibi M, Hirano T. 2000. Dissection of signaling cascades through gp130 *in vivo*: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity*, **12**, 95–105.

Pan K, Chen Y F, Roth M, Wang W B, Wang S Y, Yee A S, Zhang X W. 2013. HBP1-mediated transcriptional regulation of DNA methyltransferase 1 and its impact on cell senescence. *Molecular Cell Biology*, **33**, 887–903.

Patrik A. 2018. How to combine ChIP with qPCR. *Methods in Molecular Biology*, **1689**, 29–42.

Pittas A G, Joseph N A, Greenberg A S. 2004. Adipocytokines and insulin resistance. *The Journal of Clinical Endocrinology and Metabolism*, **89**, 447–452.

Poissonnet C M, Burdi A R, Bookstein F L. 1983. Growth and development of human adipose tissue during early gestation. *Early Human Development*, **8**, 1–11.

Poissonnet C M, LaVelle M, Burdi A R. 1988. Growth and development of adipose tissue. *The Journal of Pediatrics*, **113**, 1–9.

Rauvala H, Rouhiainen A. 2007. RAGE as a receptor of HMGB1 (Amphoterin): roles in health and disease. *Current Molecular Medicine*, **7**, 725–734.

Richard G. 2008. *Data Analysis for Experimental Design*. Guilford Press, United States.

Romao J M, Jin W, Dodson M V, Hausman G J, Moore S S, Guan L L. 2011. MicroRNA regulation in mammalian adipogenesis. *Experimental Biology and Medicine* (Maywood), **236**, 997–1004.

Rosen E D, MacDougald O A. 2006. Adipocyte differentiation from the inside out. *Nature Reviews Molecular Cell Biology*,

**7**, 885–896.

Ross S E, Hemati N, Longo K A, Bennett C N, Lucas P C, Erickson R L, MacDougald O A. 2000. Inhibition of adipogenesis by Wnt signaling. *Science*, **289**, 950–953.

Sampson E M, Haque Z K, Ku M C, Tevosian S G, Albanese C, Pestell R G, Paulson K E, Yee A S. 2001. Negative regulation of the Wnt-β-catenin pathway by the transcriptional repressor HBP1. *EMBO Journal*, **20**, 4500–4511.

Schaap F G, van der Vusse G J, Glatz J F. 2002. Evolution of the family of intracellular lipid binding proteins in vertebrates. *Molecular and Cellular Biochemistry*, **239**, 69–77.

Stros M, Launholt D, Grasser K D. 2007. The HMG-box: A versatile protein domain occurring in a wide variety of DNA-binding proteins. *Cellular and Molecular Life Sciences*, **64**, 2590–2606.

Sun Y N, Gao Y, Qiao S P, Wang S Z, Duan K, Wang Y X, Li H, Wang N. 2014. Epigenetic DNA methylation in the promoters of peroxisome proliferator-activated receptor γ in chicken lines divergently selected for fatness. *Journal of Animal Science*, **92**, 48–53.

Tevosian S G, Shih H H, Mendelson K G, Sheppard K A, Paulson K E, Yee A S. 1997. HBP1: A HMG box transcriptional repressor that is targeted by the retinoblastoma family. *G*enes *and Development*, **11**, 383–396.

Wang H, Tao Z N, Feng M, Li X T, Deng Z T, Zhao G Z, Yin H R, Pan T Z, Chen G L, Feng Z B, Li Y Y, Zhou Y X. 2020. Dual PLK1 and STAT3 inhibition promotes glioblastoma cells apoptosis through MYC. *Biochemical and Biophysical Research Communications*, **533**, 368–375.

Wang W, Zhang T M, Wu C Y, Wang S S, Wang Y X, Li H, Wang N. 2017. Immortalization of chicken preadipocytes by retroviral transduction of chicken TERT and TR. *PLoS ONE*, **12**, e177348.

Wang Y, Mu Y, Li H, Ding N, Wang Q, Wang Y, Wang S, Wang N. 2018. Peroxisome proliferator-activated receptor-*γ* gene: a key regulator of adipocyte differentiation in chickens. *Poultry Science*, **87**, 226–232.

Watanabe N, Kageyama R, Ohtsuka T. 2015. Hbp1 regulates the timing of neuronal differentiation during cortical development by controlling cell cycle progression. *Development*, **142**, 2278–2290.

Wu R F, Guo G Q, Bi Z, Liu Y H, Zhao Y L, Chen N N, Wang F Q, Wang Y Z, Wang X X. 2019. m(6)A methylation modulates adipogenesis through JAK2-STAT3-C/EBP beta signaling. *Biochimica et Biophysica Acta Gene Regulatory Mechanisms*, **1862**, 796–806.

Zhang H, Liang Q Q, Wang N, Wang Q G, Leng L, Mao J, Wang Y X, Wang S Z, Zhang J Y, Liang H, Zhou X, Li Y M, Cao Z P, Luan P, Wang Z P, Yuan H, Wang Z Q, Zhou X M, Lamont S J, Da Y, *et al*. 2020. Microevolutionary dynamics of chicken genomes under divergent selection for adiposity. *iScience*, **23**, 101193.

Executive Editor-in-Chief LUO Xu-gang
Managing Editor ZHANG Juan