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# Increased histone H3 acetylation inhibit the inflammatory response and activate the serum immunity of Pearl oyster *Pinctada fucata martensii*

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To produce cultured pearls, a mantle graft with a nucleus is transplanted into a host pearl oyster, this process is called “transplantation”. The immune response of pearl oyster after transplantation is a major factor that leads to nucleus rejection and death. Butyrate is a histone deacetylase (HDAC) inhibitor which can inhibit the deacetylation process of histones and effectively reduce the inflammatory response. To clarify the function of histone acetylation in immune response after transplantation, butyrate (10 mmol/L) was used for the treatment of pearl oysters before transplantation. Results showed that the proportion of histone H3 acetylation of the hemocytes was significantly increased after butyrate treatment before transplantation (BH group) compared with the control group at 6–24 h. Transcriptome analysis showed that butyrate treatment activated the “lysosome”, inhibited cell migration and cell proliferation at 6 and 12 h, respectively, and activated the intracellular immune recognition response of pearl oyster at 24 h after transplantation. The apoptosis detection revealed no significant difference in the proportion of apoptotic cells between the control and BH group. Moreover, butyrate treatment increased the activity of some immune-related enzymes in the serum of pearl oyster after transplantation.

## KEYWORDS

*Pinctada fucata martensii*, histone acetylation, transplantation, inflammatory response, butyrate

# 1 Introduction

Pearl oyster *Pinctada fucata martensii* is one of the most important species for pearl production in southern China. During pearl production, the donor pearl oyster is required for the preparation of mantle graft, which is implanted together with one nucleus into the host pearl oyster (Zhao et al., 2012; Wang et al., 2019). This process, which is called transplantation surgery, can induce the immune response in the host oyster, and overwhelming immune response will result in the rejection of the nucleus or failure development of pearl sac or death of host oyster (Wang et al., 2019). Different omics have been used for the analysis of the immune response of hemocyte, and many crucial pathways and genes have been found after transplantation. A large number of immune-related pathways were enriched, and immune-related genes were upregulated at 6–48 h after transplantation, suggesting that the 6–48 h after transplantation may be a critical period for immune rejection in pearl oysters (Jiao et al., 2019; Cao et al., 2021). How to reduce the excessive immune response of pearl oyster and increase its immune tolerance in the early stage of transplantation remain unclear.

Histone acetylation is one of the most important histone modifications and is an important regulatory modality that regulates the expression of inflammation-related genes and affects the onset and development of inflammation. Studies have shown that acetylation modifications can occur on four histones (H2A, H2B, H3, and H4) (Wang et al., 2012), with acetylation of histone H3 and H4 being the most common (Turner, 1991). Many sites on histone H3 can be modified by acetylation, such as K9, K14, K18, K23 and K27, acetylation of different sites can have different impacts. For example, the localization of nucleosomes is importantly associated with acetylation of the H3K9 site. In addition, acetylation of most loci is involved in gene transcription (Roth and Allis, 1996; Zhang et al., 1998). It was found that acetylation modifications of histone H3 can participate in the inflammatory response by regulating ATP-dependent chromatin remodeling (Chatterjee et al., 2011). Histone H4 acetylation is involved in chromatin transcription and chromatin replication, and the four acetylated lysine of histone H4 are specific for chromatin activity. H4 acetylated on lysine 8 or lysine 16 is preferentially recovered in the “free” histone pool, while acetylation on lysine 5 or lysine 12 is stably bound to chromatin (Mocquard-Bucher et al., 2014).

Histone acetylation and deacetylation are catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC), respectively, and regulate a broad and complex array of physiological processes, such as cell differentiation, proliferation, metabolism, senescence, and immune and apoptosis (Bai et al., 2008; Villagra et al., 2008; Wang et al., 2009). Sirt1 is a NAD<sup>+</sup>-dependent histone deacetylase, and Sirt1 deacetylates and inhibits FoxO1-dependent apoptosis (Motta et al., 2004). Sirt1 can control the self-renewal and proliferation of mouse embryonic stem cells by regulating the deacetylation of p53 (Han et al., 2005). HDAC4 interacts with the nucleotide excision repair factor XPC, which plays an important role in effectively removing the ultraviolet B-induced DNA damage (Li et al., 2020). HDAC6 plays a crucial role in the innate immune response to intracellular bacterial infection through Toll-like receptor-mediated signaling (Ran and Zhou,

2019). HDAC is associated with the pathogenesis of multiple inflammatory diseases, and HDAC inhibitors (HDI) is a potential therapeutic strategy (Adcock, 2007; De Zoeten et al., 2011).

HDI are a class of compounds, which can interfere with the function of HDAC. It has the characteristics of low toxicity and high specificity (Chuang et al., 2013), improves cell survival and increases the anti-inflammatory response (Kim et al., 2007). HDI is widely used in clinical treatment by increasing the acetylation level of histone and non-histone (Zhang et al., 2015). Butyrate, which is a commonly used HDI, is an important component of the intestinal environment and an apoptosis inducer (Pant et al., 2016), can inhibit HDAC activity, its mode of action is random and non-specific, and can lead to histone H3 and H4 hyperacetylation (Archer and Hodin, 1999). Aside from providing nutrients for intestinal epithelial cells as an energy substance, butyrate is also the main metabolite of intestinal bacteria and has a direct immune regulation impact (Semann et al., 2000). Low concentrations of butyrate can activate the BAX/BCL2 pathway, stimulate tumor cell apoptosis, and exert an anti-tumor proliferation impact (Encarncao et al., 2018). In vertebrates, butyrate affects cell growth through cell cycle arrest (Waby et al., 2010), inhibit the production of pro-inflammatory cytokines (IL-6, IL-12) (Weber and Kerr, 2006), promote the production of anti-inflammatory cytokines (IL-10) (Lee et al., 2017), and regulate the immune system to the colon mucosa maintained in a non-inflammatory state (Nancy et al., 2002). Butyrate also regulates the formation of rat beta-defensin by inhibiting TLR expression, thereby suppressing pro-inflammation upon LPS challenge (Dou et al., 2022). The function of histone acetylation was not clear in the molluscan. In the present study, butyrate was used to treat the pearl oysters before transplantation, and hemocytes were collected at 6, 12, 24 and 48 h after transplantation to construct transcriptome. The impact of butyrate on the immune response of pearl oyster after transplantation was analyzed in detail. This study aimed to determine the mechanism of histone acetylation in the immune regulation of the pearl oyster *P. f. martensii* and to provide insight into the mechanism of immune regulation during pearl production.

## 2 Materials and methods

### 2.1 Experimental design and sample preparation

The Pearl Oyster *P. f. martensii* (approximately 1.5 years old) used in this study was obtained from the sea area of Dajing village, Xuwen County, Guangdong Province. 80 pearl oyster was randomly divided into two groups - one group (BH group) was injected with 100  $\mu$ L of butyrate (Sangon Biotech, China, 10 mmol  $\times$  L<sup>-1</sup>) (Chakravorty et al., 2000), while the other group (PH group) was injected with 100  $\mu$ L of PBS (Sangon Biotech, China). All pearl oyster were injected through the adductor muscles and were transplanted immediately after the injection treatment. After transplantation, the pearl oysters were cultured in a tank containing filtered seawater. The hemolymphs of pearl oysters from the BH and PH group were collected from adductor muscles at different times (6, 12, 24, and 48 h) by using 2-mL syringes, the hemolymph was centrifuged to obtain hemocytes

and serum (4°C, 3,000 rpm, 5 min), and some hemocytes were used to extract the total histone. TRIzol reagent (Invitrogen, USA) was added to the hemocytes, and then stored at -80°C until use. Some hemocytes were fixed with paraformaldehyde (Sangon Biotech, China), and serums were stored by liquid nitrogen quick freezing.

## 2.2 Acetylation level of H3 and H4

Total histone was extracted from *P. f. martensii* of the hemocytes and EpiQuik™ total histone extraction kit (Epigentek, USA), and protein concentration was analyzed using a modified BCA protein assay kit (Sangon, China). Each group collected three hemocytes for the acetylation levels of H3 and H4 at different times after injection and transplantation, and acetylation levels were calculated based on the manual of EpiQuik™ Total Histone H3 Acetylation Detection Fast Kit and EpiQuik™ Total Histone H4 Acetylation Detection Fast Kit (Colorimetric, Epigentek, USA).

## 2.3 Total RNA extraction

Total RNA was extracted from the hemocytes of *P. f. martensii* at 6–48 h after transplantation with TRIzol reagent (Invitrogen, USA), five hemocytes in each group were collected and validated as described by Yang et al. (2018).

## 2.4 Data analysis of RNA-seq

This project used the DNBSEQ platform for sequencing and performs quality control on the raw reads data obtained. After the quality control was qualified, the data were compared with the reference genome of *P. f. martensii* using Bowtie2, the gene expression levels of each sample were also calculated using RSEM, and the genes were quantified and subjected to principal component, GO function, KEGG pathway significant enrichment, and other analyses.

## 2.5 Differential expression analysis

The DEG-Seq method allows differentially expressed genes (DEGs) to be detected. The threshold values for significant differential expression were  $FDR \leq 0.001$  and  $[\log_2 \text{Ratio (BP/SP)}] > 1$ . GO function and KEGG pathway analyses were carried out based on DEGs, and enrichment analysis was performed using the phyper function in R software to calculate the p-value, which was usually considered significantly enriched with Q value < 0.05. DEGs were analyzed using GO function and KEGG pathway (Q value < 0.05).

## 2.6 Biochemical measurements

Each group involves the collection of three serums at different times after injection and transplantation. Leucine arylamidase (LAP), catalase (CAT), phenol oxidase (PO), lysozyme (LYS), alkaline

phosphatase (ALP), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were measured according to the instruction using kits (Beijing Dongge Weiye Technology Co., Ltd., Beijing, China). Meanwhile, we also analyzed the expression of all CAT, ALP, LYS, GSH-Px in the transcriptome at different time after injection and transplantation and used the mean values to represent the expression trend.

## 2.7 Determination of cell apoptosis

The hemocyte was fixed on polylysine-treated glass slides, fixed with 4% paraformaldehyde for 1 h, and then rinsed with PBS. Blocking solution was added, and the sample was incubated for 10 min at 37°C. Permeabilizer solution was added, and the solution was incubated for 2 min at 4°C. Approximately 50 μL of labeling solution (without end transferase) was added to the cells after fixation and permeabilization as a negative control. Fixed and permeabilized cells were treated with DNase I, and 50 μL of TUNEL reaction solution was added as a positive control. The slides were incubated at 37°C for 1 h in the dark, rinsed thrice with PBS, and examined by microscopy. Five different fields of view were randomly selected for photographic counting under white light to detect apoptotic staining in different groups.

## 2.8 Data analysis

All data analyses were performed using SPSS for Windows, and  $P < 0.05$  was considered a significant difference. Differences in the levels of histone H3 and H4 acetylation, cell apoptosis and enzyme activities in different groups at the same time point were determined using t-test.

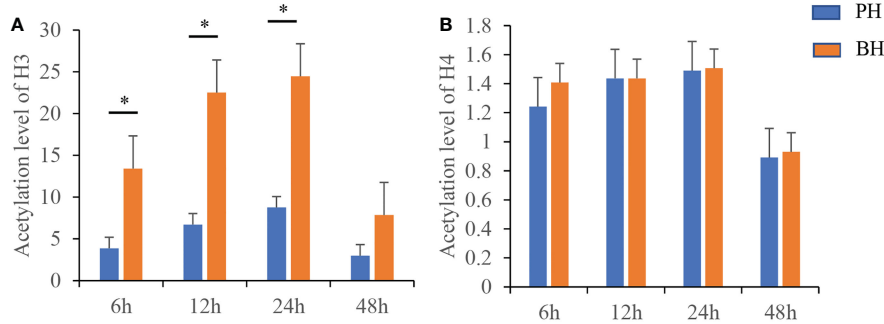
# 3 Result

## 3.1 Butyrate increased the acetylation level of H3

As shown in Figures 1A, B, the acetylation levels of H3 and H4 in the PH and BH group exhibited an increase at 6–24 h and a decrease at 48 h. In comparison with the PH group, the acetylation level of H3 in the BH group was significantly increased at 6–24 h, whereas no significant impact was found in the acetylation level of H4 at 6–48 h.

## 3.2 Transcriptome sequence assembly

At different times (6–48 h) after injection and transplantation, eight transcriptomes from two groups were successfully constructed and sequenced. Basic analysis data for each group of samples are shown in Table 1. Each sample produced an average of 21.34 M data. The comparison rates of the gene and genome were 32.3%–36.78% and 70.06%–71.45%, respectively. The quality assessment of sequencing data resulted in Q20 of 98.19%–99.02% and Q30 of 93.90%–96.49% for each sample, suggesting that the sequencing quality can be effectively used for further analysis (Table 1).



**FIGURE 1** Changes of histone H3 and H4 acetylation at different time points after butyrate stimulation. (A) the level of Histone H3 acetylation in the PH/BH group. (B) the level of Histone H4 acetylation in the PH/BH group. Blue is the PH group and red represents the BH group. Values were shown as mean ± SD (N = 3), \*P < 0.05.

### 3.3 DEG analysis

#### 3.3.1 Butyrate activated the lysosome signaling pathway and inhibited cell migration at 6 h after transplantation

In comparison with the PH group, 3,160 DEGs (FDR<0.001, fold change (FC)≥2) were obtained in the BH group at 6 h, among which 1,454 genes were significantly highly expressed, and 1,706 genes were significantly downregulated in the BH group (Figure 2A). These DEGs were mainly enriched in 61 signaling pathways by KEGG analysis (Q value<0.05), such as “Lysosome”, “Adherents junction”, “Regulation of actin cytoskeleton”, and “Focal adhesion” (Figure 2B). In the lysosome, most protease-related genes such as cathepsins (cathepsin A, C, F, L, X) were upregulated in the BH group. Thus, the function of protein degradation in lysosomes was upregulated after the butyrate treatment and transplantation. Some sugar and lipid metabolism-related genes, such as N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase (NAGPA), arylsulfatase B (ARSB), arylsulfatase I/J (ARSI/J), and alpha-N-acetylgalactosaminidase (NAGA) were upregulated in the BH group. This result indicates that butyrate treatment activates the lysosome signaling pathway (Figure 2D). Five β-actin and four α-integrin were found in the “Adherents junction”, “Regulation of actin cytoskeleton”, and “Focal adhesion” (Figures 2E–G), all of which were

significantly downregulated in the BH group, indicating that butyrate treatment inhibited cell migration. A total of 163 items were significantly enriched by GO analysis (Q value< 0.05), such as “serine-type carboxypeptidase activity” and “catalytic activity” (Figure 2C), which were related to the regulation of enzyme activity and corresponded to the enrichment of KEGG functions.

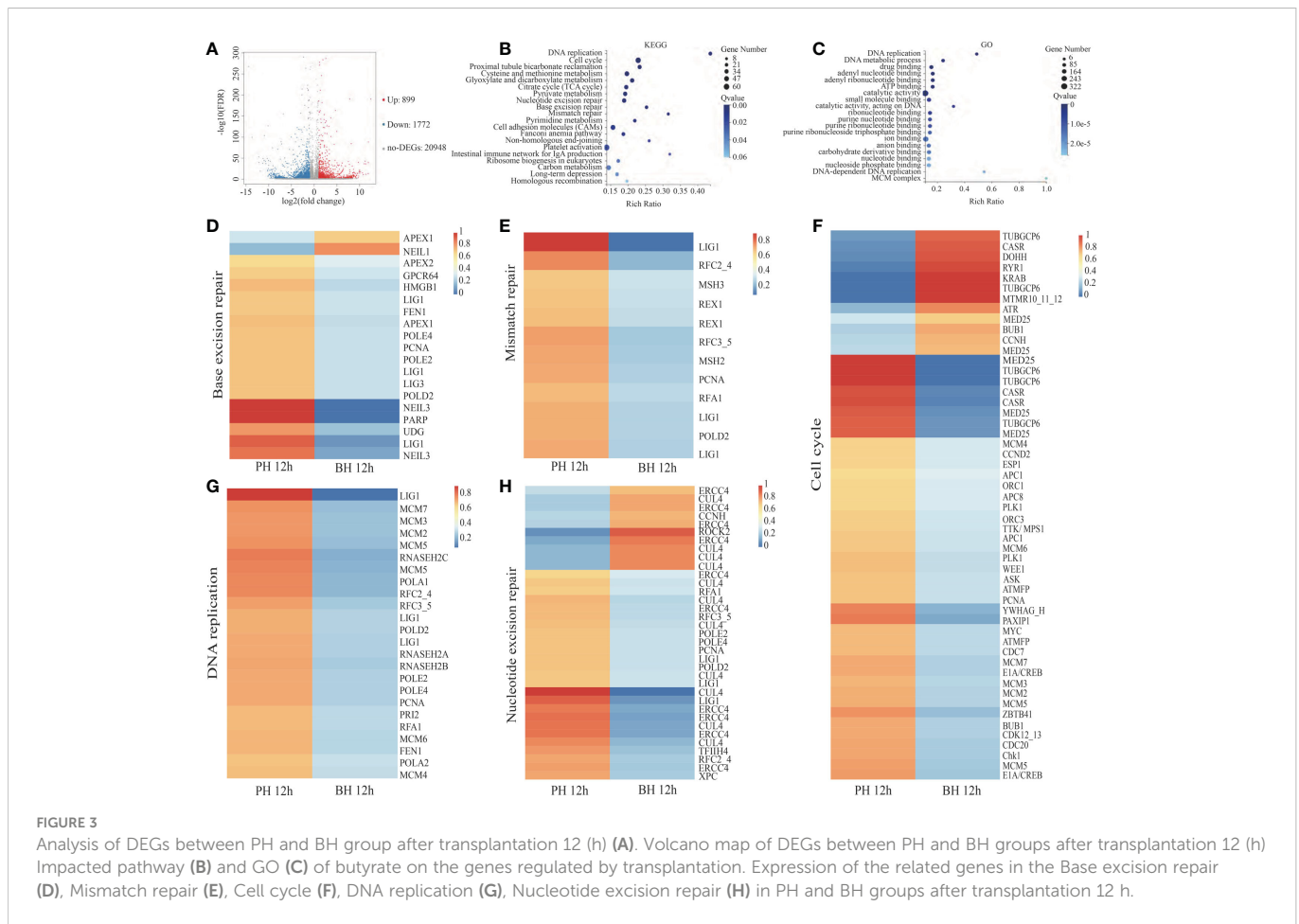
#### 3.3.2 Butyrate inhibited cell proliferation at 12 h after transplantation

In comparison with the PH group, 2,671 DEGs (FDR <0.001, fold change (FC) ≥2) were acquired in the BH group at 12 h, of which 899 genes were significantly highly expressed, and 1,772 genes were significantly downregulated in the BH group (Figure 3A). These DEGs were mainly enriched in 19 signaling pathways by KEGG analysis (Q value<0.05), such as “Base excision repair”, “DNA replication”, “Mismatch repair”, “Cell cycle”, and “Nucleotide excision repair” (Figure 3B). These pathways were associated with cell proliferation and mismatch repair, and most of the related genes in the pathway were significantly downregulated in the BH group, indicating that butyrate treatment inhibited cell proliferation (Figures 3D–H). As shown in Figure 3C, 85 items were significantly enriched by GO analysis (Q value< 0.05), such as “DNA replication” and “Cell cycle”, which correspond to the enrichment of KEGG functions.

**TABLE 1** Transcriptome data statistics.

Samples	Total Clean Reads (M)	Total Mapping to the gene (%)	Total Mapping to the genome (%)	Q20 (%)	Q30 (%)
BH-6 h	21.03	35.76	70.06	99.02	96.49
BH-12 h	21.39	36.67	70.25	98.3	94.05
BH-24 h	21.38	34.24	70.19	98.32	94.18
BH-48 h	21.39	36.78	70.62	98.29	94.03
PH-6 h	21.38	34.68	70.47	98.19	93.9
PH-12 h	21.39	36.02	71.45	98.33	94.21
PH-24 h	21.39	32.78	70.81	98.3	94.2
PH-48 h	21.39	32.3	70.86	98.26	94.04





baculoviral IAP repeat-containing protein 2/3 (BIRC2/3) and 1 Bcl-2 (Bcl-2-like 1), of which only one BIRC2/3 was significantly under-expressed, and the remaining were overexpressed in BH group. Four caspase (Cas) genes were found, of which one Cas gene was significantly overexpressed, and three were under-expressed in the BH group. In the TNF and NF-kappa B signaling pathway, most of DEG genes were apoptosis-suppressing and anti-inflammatory genes. Among these genes, one TNF receptor-associated factor 3 (TRAF3) and two NF-kappa-B inhibitor alpha (NFKBIA) were upregulated in the BH group (Figures 4G–H). Most of interleukin 17 (IL17) genes were downregulated in the BH group (Figures 4I). These results indicate that butyrate treatment activates the intracellular immune recognition response of pearl oyster, activates apoptosis, NF-κB and TNF signaling pathway, but still play a role in inhibiting the inflammatory response.

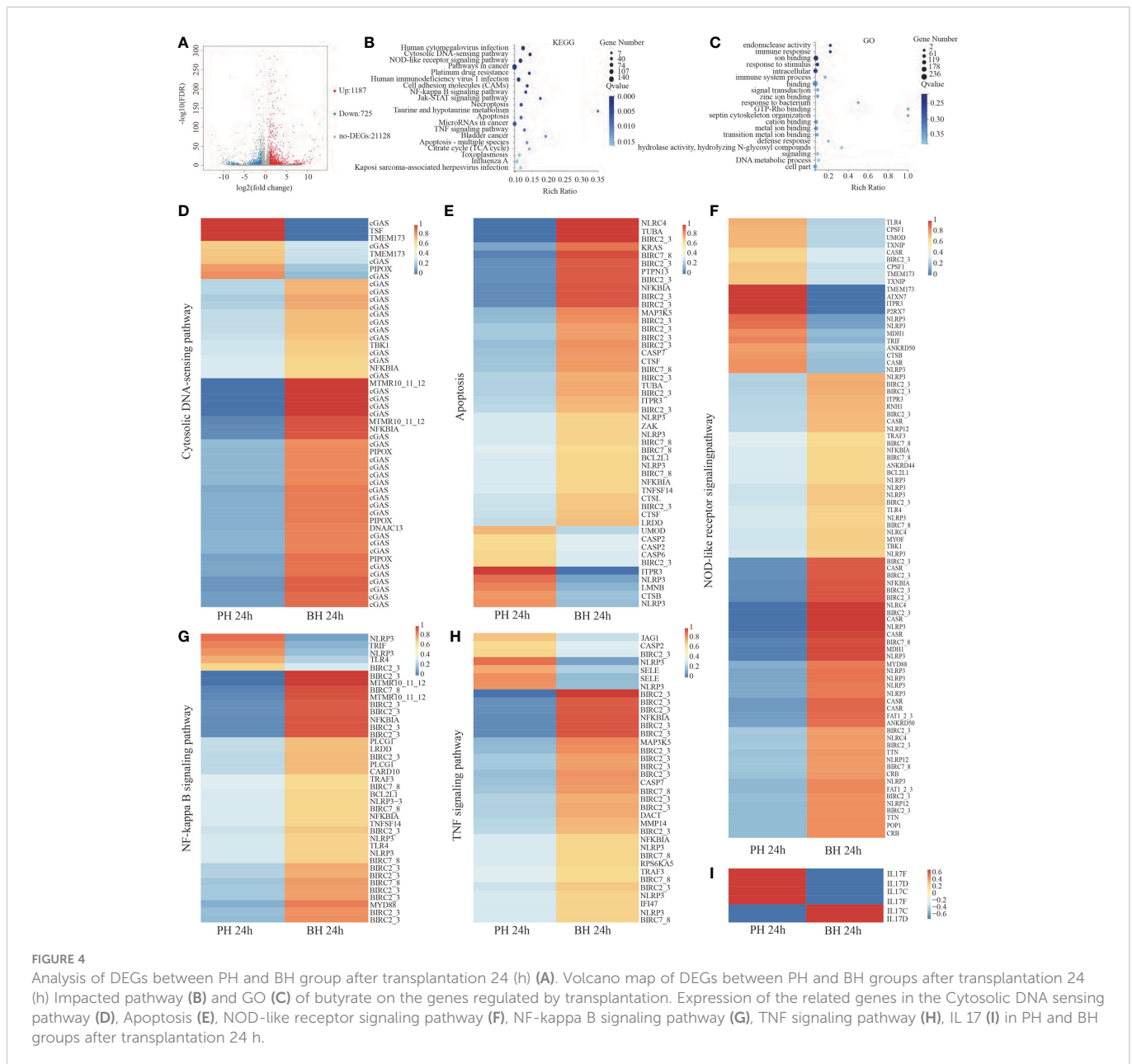
As shown in Figure 4C, 93 items were significantly enriched by GO analysis (*Q value* < 0.05), such as “response to stimulus”, “immune response”, and “defense response”, which corresponded to the enrichment of KEGG functions.

The staining of apoptotic cells in the PH and BH group was detected, five different fields of view were randomly selected, and photos were obtained and counted under white light in the same field of view, as shown in Figure 5. The apoptosis in each field of view was calculated. Statistical analysis of the proportion of apoptotic cells and SPSS analysis (*P* < 0.05) showed that the average apoptotic proportion of hemocytes in the BH group at 24 h was 0.2757%, while that in the PH group was 0.2828%. Apoptosis ratio did not significantly differ.

### 3.3.4 The impact of butyrate diminished at 48 h after transplantation

In comparison with the PH group, 3,113 DEGs (FDR < 0.001, fold change (FC) ≥ 2) were obtained in the BH group at 48 h, of which 1,595 genes were significantly highly expressed, and 1,518 genes were significantly down-expressed in the BH group (Supplementary Figure 1). These DEGs were mainly enriched in 149 signaling pathways by KEGG analysis (*Q value* < 0.05), such as “Cytosolic DNA-sensing pathway”, “NF-kappa B signaling pathway”, “Apoptosis”, “NOD-like receptor signaling pathway”, and “TNF signaling pathway” (Supplementary Figure 1).

In the cytosolic DNA-sensing pathway, 30 cGAS were upregulated in the BH group, 41 NLRs were enriched in the NOD-like receptor signaling pathway, including 29 NLRP3 and 12NLRC4, nine NLRP3 and two NLRC4 were significantly down-regulated, and the remaining genes were significantly upregulated in the BH group. In the apoptosis pathway, 24 BIRC2/3 were found, of which only 11 BIRC2/3 were significantly under-expressed, and the remaining were over-expressed in the BH group. Six Cas genes were found, of which three Cas genes were significantly over-expressed, and three were under-expressed in the BH group. In the TNF and NF-kappa B signaling pathway, one TRAF3 and one NFKBIA gene were upregulated in the BH group. The results indicate that at 48 h after transplantation, genes associated with intracellular immune recognition responses remain active in the BH group, but the differences in apoptosis and NF-κB signaling pathway between the



**FIGURE 4** Analysis of DEGs between PH and BH group after transplantation 24 (h) (A). Volcano map of DEGs between PH and BH groups after transplantation 24 (h) Impacted pathway (B) and GO (C) of butyrate on the genes regulated by transplantation. Expression of the related genes in the Cytosolic DNA sensing pathway (D), Apoptosis (E), NOD-like receptor signaling pathway (F), NF-kappa B signaling pathway (G), TNF signaling pathway (H), IL 17 (I) in PH and BH groups after transplantation 24 h.

two groups were narrowed (Supplementary Figure 1). As shown in Supplementary Figure 1, 17 items were significantly enriched by GO analysis ( $Q$  value < 0.05), such as “regulation of programmed cell death”, “regulation of cell death”, and “regulation of apoptotic process”, which were consistent to the enrichment of KEGG functions.

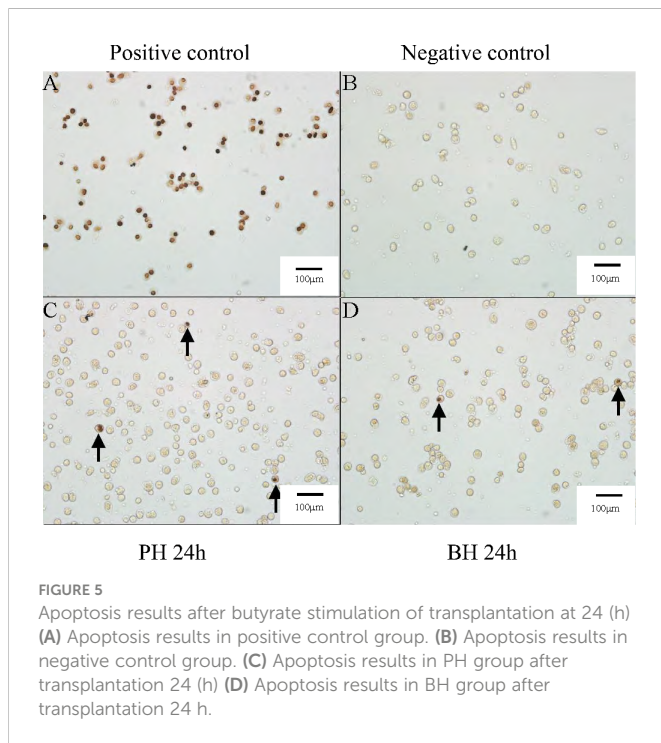
### 3.4 Butyrate treatment regulate oxidative stress

A total of one CAT, six ALP, nine GSH-Px, and two LYS genes were identified in the transcriptome and their expression trends were analyzed as the mean of the expression of each gene. Compared with those in the PH group, the expression of CAT and ALP was up-regulated at 6 h and 48 h in the BH group, the expression of LYS was up-regulated at 6 h, 24 h, 48 h, the expression of GSH-PX was up-

regulated at 6 - 48 h (Supplementary Figure 2), the activities of LAP, CAT, PO, and SOD remarkably increased at 6 h in the BH group, and the activities of LAP, CAT, PO, ALP, MDA, and SOD were significantly increased at 12 h in the BH group. All eight enzymes were significantly upregulated at 24 h in the BH group. The activities of ALP, MDA, GSH-Px, and SOD were significantly increased at 48 h in the BH group (Figure 6). These results suggest that butyrate stimulation enhances the activity of serum immunity in *P. f. martensii*.

## 4 Discussion

During producing pearls, the transplanted mantle and nucleus could induce the immune response of pearl oyster, and excessive immune response can cause nuclear rejection and death of pearl oysters. Our previous research showed that the H3 acetylation level of



**FIGURE 5**  
Apoptosis results after butyrate stimulation of transplantation at 24 (h) (A) Apoptosis results in positive control group. (B) Apoptosis results in negative control group. (C) Apoptosis results in PH group after transplantation 24 (h) (D) Apoptosis results in BH group after transplantation 24 h.

the hemocytes was significantly increased after transplantation compared with the un-transplanted pearl oysters, the HAT and HDAC genes were conserved and have a high level of expression in the hemocytes of *P. f. martensii*, and some HAT and HDAC show dynamic changes after transplantation, indicating that HAT and HDAC were involved in the immune response after transplantation. In vertebrate, butyrate was reported to induce the relaxation of the chromatin structure, provide better access to transcription-related proteins (Jazirehi, 2010), and have an important role in suppressing inflammation (Jiang et al., 2021). In the present study, butyrate was used to treat pearl oysters before transplantation, and hemocytes were collected at 6, 12, 24 and 48 h after transplantation to construct transcriptome. The effect of histone acetylation on the immune response of pearl oyster after transplantation was analyzed in detail.

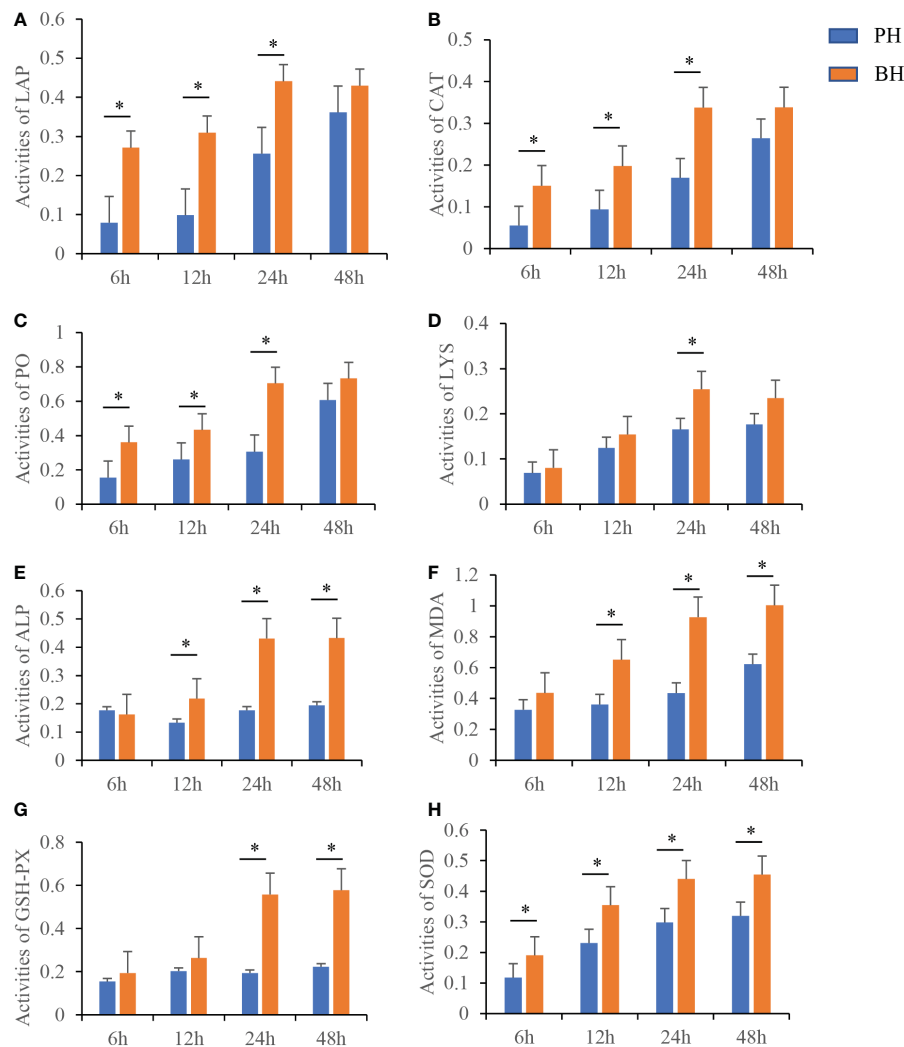
Our results showed that the level of histone H3 acetylation in the hemocytes of BH group was significantly increased compared with PH group, indicating that the state of histone acetylation modification changed after butyrate stimulation. After transplantation for 6 h, the BH group had 3160 DEGs compared with the PH group. Functional enrichment analysis showed that “Lysosome”, “Adherents junction”, “Regulation of actin cytoskeleton” and “Focal adhesion” were significantly enriched. Lysosome, an intracellular digestive organ, is involved in cellular degradation and waste recycling, and it plays an important role in the phagocytosis and degradation of pathogens. Furthermore, lysosomes participate in some fundamental processes, such as secretion, energy and signaling metabolism, and plasma membrane repair (Settembre et al., 2013). This study showed that lysosomal enzyme-related genes, such as cathepsin and arylsulfatase, were significantly up-regulated in the BH group, indicating that butyrate treatment activates the lysosome signaling pathway, which may help the pearl oyster to deal with the damaged cellular organs or cells. The same conclusion was also reported in HeLa cells, which underwent morphological differentiation after treatment with

butyrate, and increased lysosomal enzyme activity (Kelly, 1985). In addition, focal adhesions and adherens junctions are associated with cell migration (Cohen et al., 2013). Inflammatory caspases can regulate cell migration through actin remodeling (Papoff et al., 2018). This study showed that  $\beta$ -actin and  $\alpha$ -integrins were downregulated in the BH group, integrins can participate in phagocytosis and attachment of hemocytes (Terahara et al., 2006; Lv et al., 2020). Thus, lysosomal signaling pathways, Cell repair and cell migration may be a potential way in which butyrate regulates immunity in pearl oyster after transplantation.

Eukaryotic cells respond to immune stimulation by activating signaling pathways that promote cell cycle arrest and DNA repair. Previous studies have found that the expression of genes related to “cell cycle”, “DNA replication” and “DNA mismatch repair” is up-regulated after transplantation (Jiao et al., 2019). Our data also showed that these DEGs were significantly enriched in cell proliferation-related pathways, while most genes in the pathway were downregulated in the BH group after transplantation 12 h. It was shown that the cell cycle of HEK293, HeLa and MCF-7 cell was arrested by butyrate, and that cell cycle arrest may be one of the main reasons for the proliferation-inhibiting impact of butyrate (Li et al., 2015). Therefore, butyrate could suppress inflammatory responses by inhibiting hemocytes proliferation after transplantation.

After transplantation 24 h, 1,912 differential genes were observed between the BH and PH group. The results of functional enrichment analysis illustrated that these DEGs were enriched in cytosolic DNA-sensing, NOD-like receptor signaling, NF- $\kappa$ B signaling, and TNF signaling pathway and apoptosis. cGAS can recognize the cytoplasmic and exogenous DNA fragments (e.g., viruses, bacteria, and parasites) and its own damaged DNA (e.g., damaged mitochondrial and micronuclear DNA (Mackenzie et al., 2017). NLRP3 and NLRC4 in the NOD-like receptor signaling pathway are also intracellular pattern recognition receptors. They can recognize various foreign pathogens (e.g., bacteria, viruses, and fungi) and can be activated by their own “danger signals”. For example, ATP and urate are released when host cells are damaged and necrotic (Jin and Flavell, 2010); In the present study, cGAS, NLRP3, and NLRC4 are upregulated with butyrate treatment before transplantation, suggesting that the activation of these intracellular recognition receptors may be caused by butyrate-induced cell damage. As an anti-inflammatory factor, TRAF3 participates in the process of cellular immune regulation and can inhibit the NF- $\kappa$ B signaling pathway by continuously mediating the degradation of NIK (Huang et al., 2012) and specifically block NF- $\kappa$ B activation by TRAF2/5 (Hauer et al., 2005). NF- $\kappa$ B, as a protein transcription factor, coordinates many inflammatory processes. I $\kappa$ B $\alpha$ , is an important inhibitor of NF- $\kappa$ B activity, which is encoded by NFKBIA (Ali et al., 2013). In the present study, TNF and NF- $\kappa$ B-signaling-pathway-related genes were upregulated with butyrate treatment before transplantation, while most of DEG genes in these pathways were apoptosis-suppressing and anti-inflammatory genes. We analyzed the expression of inflammatory factor IL17 and anti-inflammatory factors TRAF3, NFKBIA and found that the expression of IL17 was mostly decreased and the expression of TRAF3 and NFKBIA were significantly upregulated with butyrate treatment before transplantation, suggesting that butyrate could inhibit the inflammatory response. Chakravorty et al. (2000) found that butyrate prevented NF- $\kappa$ B activation by stabilizing I $\kappa$ appaB- $\alpha$  and





**FIGURE 6**  
Changes of different activity levels at different time after butyrate stimulation. Activity of LAP (A), CAT (B), PO (C), LYS (D), ALP (E), MDA (F), GSH-Px (G) and SOD (H) in serum at different time points in PH and BH group after transplantation. Values were shown as mean  $\pm$  SD (N = 3), \*P < 0.05.

IkappaB- $\beta$  and inhibited the formation of nitric oxide induced by LPS in RAW 264.7 cells. These results support our data about the inhibition of NF- $\kappa$ B signal by butyrate. Moreover, the apoptosis pathway was also significantly enriched in the study, but in which a large number of apoptosis-suppressing genes were induced. And no significant difference was observed in the apoptotic cells between BH and PH group after transplantation for 24 h by apoptosis detection. This phenomenon indicated that butyrate induced the large-scale expansion of apoptosis-suppressing genes in *P. f. martensii*, thus inhibiting the occurrence of apoptosis. After transplantation for 48 h, the enriched signaling pathways were similar to those at 24 h, but the proportion of induced genes decreased, indicating that the impact of butyrate was gradually decreasing. Therefore, the cytosolic DNA-sensing, NOD-like receptor signaling, NF- $\kappa$ B signaling, and TNF signaling pathway and apoptosis may be the key pathways for the butyrate to regulate the excessive inflammatory response induced by transplantation.

To further explore the function of butyrate, we analyzed the effect of butyrate on the oxidative stress response. Results showed that the

content of MDA was significantly increased. It was found that butyrate treatment resulted in an increase in ROS levels, we proposed that the increased MDA levels may be a result of the increasing ROS induced by butyrate. [Yuksel et al. \(2022\)](#) also found an up-regulation of MDA at 48 hours after butyrate treatment. To investigate whether butyrate exerts an antioxidant effect, we analyzed the activities of enzymes such as SOD, GSH-Px and CAT, which are important enzymes that resist intracellular ROS damage, boost the defense system and protect cells from oxidative damage ([Jing et al., 2007](#); [Camini et al., 2016](#)). Previous studies have shown that the addition of butyrate to different fish ([Abdel-Latif et al., 2020](#)) and *Procambarus clarkii* ([Xiao et al., 2021](#)) diets increased the activity of antioxidant enzymes SOD, CAT and GSH-Px. [Li et al. \(2021\)](#) also found that butyrate increased antioxidant enzyme activities (CAT, SOD and GSH-Px) in the brain. This was consistent with the results of the present study, and the activities of antioxidant and immune-related enzymes were significantly increased after butyrate treatment, suggesting that butyrate may modulate the oxidative stress response of pearl oyster.

## Conclusions

This study provides an explanation for the molecular mechanism of pearl oyster immune response after butyrate stimulation. Butyrate can increase the acetylation level of H3 and regulate gene expression. The transcriptomic of the hemocytes analysis revealed that butyrate can activate the lysosome pathway and modulate transplantation-induced inflammatory responses by regulating cell proliferation in pearl oysters. “Cytosolic DNA-sensing pathway”, “NOD-like receptor signaling pathway”, “Apoptosis”, “NF-kappa B signaling pathway” and “TNF signaling pathway” may be the key pathways for butyrate to exert its regulation impacts. Moreover, butyrate treatment can enhance the activity of some immune related enzyme in the serum after transplantation. Therefore, butyrate treatment can regulate the immune response of pearl oyster and activate humoral immunity.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: CNGB, CNP0003692.

## Author contributions

YJ and YD: Conceptualization, Methodology, Writing-Reviewing and Editing. JY: Data curation, Writing- Original draft preparation. SY and YL: Sample Preparation. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1073322/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

Analysis of DEGs between PH and BH group after transplantation 48 h. (A) Volcano map of DEGs between PH and BH groups after transplantation 48 h. Impacted pathway (B) and GO (C) of butyrate on the genes regulated by transplantation. Expression of the related genes in the Cytosolic DNA-sensing pathway (D), Apoptosis (E), NOD-like receptor signaling pathway (F), NF-kappa B signaling pathway (G), TNF signaling pathway (H) in PH and BH groups after transplantation 48 h.

### SUPPLEMENTARY FIGURE 2

Expression of response to stimulus-related genes and antioxidantactivity-related genes at difference times in the transcriptomes of PHand BH group. Expression of CAT (A), ALP (B), LYS (C) and GSHPx (D) at difference times after transplantation. Blue is the PH groupand red represents the BH group.

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