Research Article

Arih2 gene influences immune response and tissue development in chicken

Guanxian Wu*, Sifan Xu*, Wanting Zhang†, Yang Liu†, Qiu yuan Wang* and Chaolai Man*

College of Life Science and Technology, Harbin Normal University, Harbin 150001, P.R. China

Correspondence: Chaolai Man (manchaolai@126.com)

Ariadne homolog 2 (ARIH2), an E3 ubiquitin ligase, is one of the important factors involved in regulating biological functions, such as inflammation and skeletal muscle degeneration. In the present study, the full-length coding sequence of Arih2 gene was cloned from Hy-Line Brown chicken. The tissue transcriptional profiles of Arih2 gene at different developmental stages were detected using quantitative real-time PCR (qRT-PCR), and the Arih2 functional characteristics in immune response were analyzed. The results showed that the full-length coding sequence of Arih2 gene was 1473 bp, encoding 490 amino acids, and conservative between different species. The Arih2 gene was transcribed in various tissues at different developmental stages, and its transcriptional activities varied significantly between multiple tissues. With the development of chicken, Arih2 gene was basically up-regulated in heart, liver, kidney, skeletal muscle and glandular stomach, but fluctuated significantly in large intestine. In immune response, the transcriptional activities of Arih2 gene exhibited significant changes in the bursa, thymus and blood (P<0.05). The results showed that Arih2 might be a multifunctional gene involved in tissue development and immune response in chicken, and have a potential possible application as diagnostic marker for identifying immune response.

Ariadne homolog 2 (ARIH2, also known as TRIAD1) belongs to a RING1-in-between-RING2 (RBR) E3 ligase family, which is a type of E3 ubiquitin ligase with RING domain and synthesis mechanism of ubiquitin chain, and plays important physiological functions in nucleus [1,2]. ARIH2 can coordinate aging-associated skeletal muscle degeneration with the muscle regulator polyadenylate-binding protein nuclear 1 (PABPN1) in mouse, and modify skeletal muscle by interacting with TsUBE2L3 in nematode [3–5]. In addition, conditional regulation of Arih2 transcription by the tyrosine phosphorylation states of HoxA9 and HoxA10 can contribute to terminating the innate immune response in mouse [6]. ARIH2 can antagonize NF-κB signaling by promoting the degradation of nuclear IκB, which can activate the NF-κB pathway and promote the occurrence of inflammatory response [1,7]. Also, deletion of endogenous Arih2 inhibits nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) ubiquitination and promotes NLRP3 inflammasome activation, whereas Arih2 overexpression promotes NLRP3 ubiquitination and inhibits its activation [8,9]. Moreover, study has confirmed that Arih2 gene is associated with inflammatory bowel disease and can become a potential therapeutic target for inflammatory diseases [10].

Since ARIH2 plays key roles in the immune response [1,6], inflammation [1,8,12] and skeletal muscle modification [4,5], it is necessary to study the functions and characteristics of chicken Arih2 gene. At present, inflammation-related diseases and growth rates are still main problems affecting poultry industry. Studying chicken Arih2 gene may provide a positive reference for solving these problems. In addition, related studies on Arih2 gene have not been reported in chicken. In the present study, the full-length coding sequence of chicken Arih2 was cloned from Hy-Line Brown chicken and analyzed. Then, the Arih2 transcriptional profiles at different developmental stages were analyzed. Finally, the possible functions of Arih2 were explored in immune response. The present study can lay the foundation for further studying Arih2 functions.
Materials and methods
Experimental animal and tissue collection
The Hy-Line Brown chickens (14-day-old, 10- and 24-month-old) were obtained from Northeast Agricultural University poultry farm. The 16 tissues (heart, liver, spleen, lung, kidney, brain, skeletal muscle, muscle stomach, thymus, skin, small intestine, large intestine, glandular stomach, fat, blood and bursa) were separated from the three different ages of chickens respectively, then frozen in liquid nitrogen and stored at −80°C.

Cloning and bioinformatics analysis of Arih2 gene
Total RNA from heart, liver, spleen, lung, kidney, brain, skeletal muscle, thymus, muscle stomach, skin, small intestine, large intestine, glandular stomach, fat, bursa and blood of chickens at the three different ages were extracted using TRIzol reagent (Sigma–Aldrich, St. Louis, MO, U.S.A.) and reverse transcribed into cDNA using FSQ-301 kit (TOYOBO, Shanghai) according to the manuals, respectively. The coding sequence (CDS) of Arih2 gene was amplified by PCR with primers based on the predicted nucleotide sequence of Arih2 from Gallus gallus (GenBank: NM_001199221), and the muscle stomach cDNA of 14-day-old chicken as a template. The primer sequences were as follows: 5′-GCTCAGATGAATCAGAAAGGCGGT-3′ and 5′-CTGCTATCCCGGTGAAACGGCAT-3′. The total PCR system was 25 μl including: 0.5 μl cDNA (20 ng/μl), 0.8 μl of each primer, 2 μl dNTP mixture (2.5 mM), 2.5 μl of 10× ExTaq buffer, 1 μl ExTaq™ DNA polymerase (Takara Bio Inc.) and 17.4 μl sterile deionized water. The PCR procedure was as follows: 95°C/5 min; 30 cycles of 94°C/1 min, 65°C/1 min, 72°C/45 s; 72°C/10 min; 4°C to terminate the reaction. The PCR products were cloned into vector PMD18-T (TaKaRa, Dalian) and identified by double digestion with restriction enzymes (BanH I and Sal I). Five positive clones with the correct identification were randomly selected and sequenced by Shanghai Bioengineering Co., Ltd. The nucleotide and amino acid sequences of Arih2 gene were analyzed using SOPMA, DNAMAN and MEGA7.0 software etc.

Transcriptional profile analysis of Arih2 gene
Quantitative real-time PCR (qRT-PCR) was used to analyze the transcriptional activities of Arih2 gene in each tissue. The qRT-PCR system doses and reaction procedures of the Arih2 gene were the same as the internal reference β-actin (GenBank number: NM205518). The primers used for qRT-PCR were as follows: target gene Arih2: 5′-AGGACATATGGAGAGCCGTACCTACC-3′ and 5′-AAGCAGAGACCTCATGGGAAGCAT-3′; internal reference gene β-actin: 5′-CCAACTGGGATGATAT-3′ and 5′-CGTACTCTTGTTGCTGAT-3′. qRT-PCR total system was 20 μl including: 10 μ l 1 × SYBR Green I (TOYOBO, Shanghai), 0.4 μ l 50× ROX reference dye (TOYOBO, Shanghai), 0.6 μ l of each primer, 2 μ l cDNA and 6.4 μ l ddH2O. The reaction procedure was as follows: 95°C/1 min; 40 cycles of 95°C/15 s, 56°C/30 s, 72°C/40 s.

Transcriptional characteristics of Arih2 gene in immune response
In order to verify whether Arih2 was involved in immune response, the 11-day-old Hy-Line Brown chickens were vaccinated with LaSota (Heilongjiang Biological Production Company, Harbin, China) according to the manufacturer’s instructions by the eye-drop method [11]. Five immune-related tissues such as blood, liver, spleen, thymus and bursa were collected on the day 14 post-vaccination. The methods of total RNA extraction, reverse transcription and qRT-PCR were the same as that in ‘Transcriptional profile analysis of Arih2 gene’ section.

Statistical analysis
In the present study, three chickens were randomly selected for qRT-PCR analysis, and each chicken was subjected to three technical repetitions. The relative transcriptional activity of Arih2 was analyzed using 2^−ΔΔCt method. The data were analyzed by one-way ANOVA using SPSS 20.0 software, and finally plotted using GraphPad Prism7.0 software.

Results and discussion
Arih2 has been shown to be widely involved in important physiological processes, such as innate immune response and inflammation [1,8,12]. In this study, the Arih2 gene was cloned and its transcriptional profiles were analyzed at different developmental stages. In order to understand the possible functions of Arih2 in adaptive immune response, the transcriptional activities of Arih2 mRNA in immune-related tissues were identified in immunized chicken.

The sequencing results showed that the coding region of Arih2 gene was 1473 bp and encoded 490 amino acids. Based on the predicted Arih2 gene of Gallus gallus, Arih2 gene was located on chromosome 12 with sixteen introns and seventeen exons, and there were four SNPs in the coding region of Hy-Line Brown chickens Arih2 gene: the
mutation points were C to T (position 786 nt), A to G (position 1198 nt) which resulted in the mutation of amino acid (position 400 AA) from threonine (T) to alanine (A), C to A (position 1441 nt), A to T (position 1466 nt) which resulted in the mutation of amino acid (position 489 AA) aspartic acid (D) to proline (V) (Figure 1). Multiple glycosylation and phosphorylation functional sites (N-glycosylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site, Protein kinase C phosphorylation site and a cell attachment site) were located in the C-terminus of ARIH2 protein, which suggested that the ARIH2 C-terminal might play an important role in the cellular localization and protein modification. Structural analysis showed that chicken ARIH2 protein had a secondary structure dominated with the C-terminal α-helices and a symbolic structure domain (RING1-IBR-RING2) of the aziandine RBR family (Figures 1 and 2). In addition, homology comparison and evolutionary analysis were carried out based on the sequences of nucleotide and amino acid of Arih2 gene coding regions among 18 species, respectively (Figure 3). The results showed that the Arih2 genes had high homology between different species, and chicken ARIH2 was in the middle position of the higher mammal and the amphibian and fish in evolution, which suggested that chicken was an excellent model animal for studying Arih2 gene.

Expression profiling analysis revealed that chicken Arih2 mRNA was transcribed in all tissues, but the transcriptional activities had differences in the same tissues at different development stages or in the different tissues at the same age (Figure 4). According to the range of relative transcriptional activity, we divided it into three levels. For example, in 14-day-old chickens, Arih2 was highly transcribed (the relative transcriptional activity value was higher than 0.006) in heart, medium transcriptional activity (the value of relative transcriptional activity was between 0.003 and 0.006) in the liver, lung, kidneys and brain, and low transcriptional activity (the value of relative transcriptional activity was below 0.003) in the spleen, muscle, stomach, small intestine, large intestine, skin, thymus, bursa and fat. In 10-month-old chickens, Arih2 was highly transcribed in the heart, liver, brain, muscle, large intestine and fat, medium transcriptional activity in the lung, kidney and small intestine, and low transcriptional activity in the other tissues; In 24-month-old chickens, Arih2 was highly transcribed in the heart, liver, lungs, glandular stomach, kidneys and muscle, and low transcriptional activity in the rest of tissues. Basically, with different stages of development, the transcriptional activity of Arih2 gene was up-regulated in many tissues (such as heart, liver, kidney, lung, brain, skeletal muscle and glandular stomach), but there was no corresponding change in a few tissues (spleen, thymus, skin, muscle stomach, fat and small intestine), suggesting that Arih2 gene might have different functions in different tissues. Studies have shown that ARIH2 regulates PABPN1 protein turnover and affects muscle cell fusion, and ARIH2 can suppress ubiquitination and degradation of skeletal muscle-specific proteins through binding TsUBE2L3, thus make muscle proteins more stable [3–5]. In this study, Arih2 was highly transcribed in heart and skeletal muscle of the three different developmental stages, which suggested that Arih2 might be involved in the positive regulation of the muscle development. Interestingly, Arih2 transcription was significantly up-regulated in the large intestine tissue of 10-month-old chickens. Studies showed that E3 ubiquitin ligases IITCH, ring finger protein 4 (RNF4) and ubiquitin fold modifier 1 (Ufm1) played important roles in maintaining intestinal homeostasis and mucosal immunity [13–15]. Arih2, as E3 ubiquitin ligase gene, had high transcriptional activity in the large intestine of 10-month-old chickens that were in the stage of rapid growth and enhanced digestibility. It was speculated that Arih2 might also participate in the proliferation and formation of defense barrier of large intestinal epithelial cells, thus maintaining the stability of the intestinal environment.

Previous study in our lab showed that the titers of antibodies were in the ascending stage at 14 days after vaccine immunization, which was the positive reaction period of acquired immune response and could better reflect the expression status and functional characteristics of target genes [11]. The results of transcriptional activity analysis showed that Arih2 was significantly up-regulated in the blood of immunized chickens (Figure 5). Erythrocytes are the most abundant blood cells in the blood and play an important role in the body's anti-infective immunity. Study showed that human HB (hHB) could enhance the RIG-I mediated antiviral responses through promoting the RIG-I ubiquitination depending on the hHB-induced reactive oxygen species (ROS) [16]. Therefore, it was speculated that the high transcriptional activity of Arih2 in blood might mainly come from erythrocytes, and ARIH2, an E3 ligase, might be involved the RIG-I ubiquitination process and participate in the anti-infective immune function of erythrocytes. In addition, chicken blood can be obtained easily, and several studies have shown that the changes of target gene mRNA levels can be used as diagnostic markers or detection indicators for tumors, cancers and myelodysplastic diseases [17–19], so the significant changes of Arih2 gene transcriptional activity in blood might become a potential diagnostic marker for identifying immune response, which might be helpful for serological antibody detection. Moreover, the transcriptional activity changes of Arih2 in the central immune organs (bursa and thymus) were opposite and different obviously. The Arih2 transcriptional activity was distinctly up-regulated in the thymus, but significantly down-regulated in the bursa. However, there were no significant transcriptional differences between the peripheral immune organs (liver and spleen) (Figure 5). Study showed that if Arih2 was absent from mice, T-cell
**Figure 1.** The complete coding sequence of Hy-Line Brown chicken Arih2 gene and its encoding amino acids

The italics indicate the start codon; the start (*) indicates the stop codon; the bold and underline indicate the SNP sites, and the bold indicates the amino acid mutation due to the base mutation; the sequences in the box represent conserved domain (RING1: 136-169AA, IBR: 205-267AA; RING2: 297-337AA). Functional sites are shaded (N-glycosylation site (348-NQSQ-351, 373-NKSL-376 and 453-NLSW-456), cAMP- and cGMP-dependent protein kinase phosphorylation sites (479-RRRT-482), Protein kinase C phosphorylation site (63-TYK-65, 81-TLK-83, 124-SSK-126, 269-TIR-271 and 455-SWK-457), Casein kinase II phosphorylation site (12-SNEE-15, 63-TYKE- 66, 70-TLNE-73, 160-SCWE-163 and 189-TPED-192), Tyrosine kinase phosphorylation site (213-RDYVESHY-220), N-myristoylation site (9-GSDSNE-14 and 173-GVGVGV-178), Cell attachment sequence (466-RGD-468), Zinc finger RING-type signature (315-CKHDFCWMCL- 324)).

© 2019 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
Figure 2. The secondary structure of chicken ARIH2 protein
The result was predicted by the SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) software, and the configurations represented by the length of the line segment from long to short are α-helix, extended chain, β-turn and random coil. The numbers represent the location of amino acids. RING1-IBR-RING2 (136-337AA).

Figure 3. Homology and evolution analysis of multiple sequences of Arih2 gene
(A) Nucleotide homology analysis of Arih2 coding sequences using DNAMAN software (http://www.Lynnon.com). Interval range of scale corresponds to homology value of homologous tree. Percentages on branches denote similarity between sequences.
(B) Phylogenetic tree analysis of ARIH2 amino acid sequences. The phylogenetic tree was obtained by bootstrap analysis with the neighbor-joining method. Numbers on the branches represent bootstrap values for 1000 replications. Scales represent indicating differences between display sequences, and 0.02 indicates that two of the 100 bases are different, and the branching value represents its bootstrap value. Human (GenBank:NM_001317333.1); Chimpanzee (GenBank:NM_001280136.1); Mouse (GenBank:NM_001357283.1); Cattle (GenBank:NM_001206242.2); Pig (GenBank:XM_003132197.6); Macaque monkey (GenBank:XM_015130961.1); Gallus gallus (GenBank:NM_001199221); Hy-Line Brown chicken (GenBank:MK_335707); Guinea fowl (GenBank:XM_021409574.1); Quail (GenBank:XM_015875271); African clawed frog (GenBank:NM_001096778); Whale shark (GenBank:XM_020527432.1); Condor (GenBank:XM_010571148.1); Mallard (GenBank:XM_027468046.1); Nanorana parkeri (GenBank:XM_018576133.1); Zebrafish (GenBank:NM_213143.1); Elephant shark (GenBank:XM_007890321.1); Tit (GenBank:XM_015640917.2).

In conclusion, we first cloned chicken Arih2 gene and analyzed transcriptional profiles. With the development of chicken, the transcriptional activity of Arih2 increased gradually in multiple tissues, and fluctuated significantly in large intestine. The transcriptional activity of Arih2 gene was up-regulated significantly in blood during immune response [1]. In the central immune organs, the significant changes of Arih2 suggested that Arih2 might be highly correlated with the differentiation and maturation of T and B cells, but the participation mechanisms might be different. The down-regulation of Arih2 transcripts in liver and spleen further enhanced immune response, because ARIH2 expression activity was negatively correlated with immune response [1,6].
Figure 4. Transcriptional levels of Arih2 gene in different developmental stages of Hy-Line Brown chicken

(A) Relative transcriptional activity analysis of Arih2 gene in 14-day-old Hy-Line Brown chicken. (B) Relative transcriptional activity analysis of Arih2 gene in 10-month-old Hy-Line Brown chicken. (C) Relative transcriptional activity analysis of Arih2 gene in 24-month-old Hy-Line Brown chicken. The letters on bars in the figure represent significant differences in transcriptional activity between tissues. The data are indicated as mean ± SD. Different letters (a–f) represent significant differences (P<0.05), while the same letters represent no significant differences (P>0.05).

Figure 5. Expression changes in Arih2 gene on the day 14 post-vaccination

The 11-day-old Hy-Line Brown chickens of experimental group were vaccinated with LaSota, while age-matched chickens in the control group were administered with PBS (20 mM phosphate, 150 mM NaCl, pH 7.4) by the same method. The data are indicated as mean ± SD. The * and ** represent the significant difference between the experimental group and control group. * indicates statistically significant (P<0.05) and ** indicates statistically highly significant (P<0.01).

response, which suggested that Arih2 gene might have a potential application value for identifying immune response. The results implied that Arih2 gene was involved in tissue development and adaptive immune response. These data can serve as a foundation for further studying Arih2 gene.
Ethics Statement
The proposed study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Harbin Normal University (No. SYXKHEI2008006). In Harbin Normal University, all animals were handled in compliance with the guidelines set forth by the Guide for the Care and Use of Laboratory Animals and according to the guidelines of the Institutional Animal Care and Use Committee. All chickens were humanely killed by an anesthetic overdose of Sumianxin II (0.2 ml/kg body weight; Academy of Military Medical Sciences, Changchun, China).

Author Contribution
Guanxian Wu: performed the experiments, analyzed the data and wrote the paper. Guanxian Wu and Sifan Xu: collection of tissue samples. Wanting Zhang, Yang Liu, and Qiuyuan Wang: contributed the reagents and materials that were used in the present study. Chaolai Man: conceived and designed the present study, and revised the paper.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was supported by the Science Foundation of Heilongjiang Province [grant number LH2019C073]; and the Pre-research Project of Harbin Normal University [grant number 12XYG-08].

Abbreviations
ARIH2, Ariadne homolog 2; HB, Hemoglobin; hHB, human HB; HoXA, HomeoboxA; IκBβ, Inhibitor of kappa B-beta; NLRP3, nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3; PABPN1, polyadenylate-binging protein nuclear 1; qRT-PCR, quantitative real-time PCR; RIG-I, Retinoic acid inducible gene I.

References
1 Lin, A.E., Ebert, G., Ow, Y., Preston, S.P., Toe, J.G., Cooney, J.P. et al. (2013) ARIH2 is essential for embryogenesis, and its hematopoietic deficiency causes lethal activation of the immune system. Nat. Immunol. 14, 27–33, https://doi.org/10.1038/ni.2478
2 Duda, D.M., Oliszewski, J.L., Schuermann, J.P., Kurinov, I. and Miller, D.J. (2013) Nourse A. Structure of HHARI, a RING-IBR-RING ubiquitin ligase: autoinhibition of an Ariadne-family E3 and insights into ligation mechanism. Structure 21, 1030–1041, https://doi.org/10.1016/j.str.2013.04.019
3 Rao, P., Hayden, M.S., Long, M., Scott, M.L., West, A.P., Zhang, D. et al. (2010) IkappaBbeta acts to inhibit and activate gene expression during the inflammatory response. Nature 466, 1115–1119, https://doi.org/10.1038/nature09283
4 Kawashima, A., Karasawa, T., Tago, K., Kimura, H., Kamata, R., Usui-Kawanishi, F. et al. (2017) ARIH2 ubiquitinates NLRP3 and negatively regulates NLRP3 inflammasome activation in macrophages. J. Immunol. 199, 3614, https://doi.org/10.4049/jimmunol.1700184
5 Wang, H., Wang, Y., Du, Q., Lu, P., Fan, H. and Lu, J. (2016) Inflammasome-independent nlrp3 is required for epithelial-mesenchymal transition in colon cancer cells. Exp. Cell Res. 342, 184–192, https://doi.org/10.1016/j.yexcr.2016.03.009
6 Prescott, N.J., Lehne, B., Stone, K., Lee, J.C., Taylor, K., Knight, J. et al. (2015) UK IBD Genetics Consortium. Pooled Sequencing of 531 genes in inflammatory bowel disease identifies an associated rare variant in BTNL2 and implicates other immune related genes. PLoS Genet. 11, e1004955, https://doi.org/10.1371/journal.pgen.1004955
7 Lin, A.E., Ebert, G., Ow, Y., Preston, S.P., Toe, J.G., Cooney, J.P. et al. (2013) ARIH2 is essential for embryogenesis, and its hematopoietic deficiency causes lethal activation of the immune system. Nat. Immunol. 14, 27–33, https://doi.org/10.1038/ni.2478
8 Wang, H., Be, L., Shah C, A., Hu, L. and Eklund, E.A. (2015) HoxA10 terminates emergency granulopoiesis by increasing expression of triad. J. Immunol. 194, 5375–5387, https://doi.org/10.4049/jimmunol.1401909
9 Wang, H., Be, L., Shah C, A., Hu, L. and Eklund, E.A. (2015) HoxA10 terminates emergency granulopoiesis by increasing expression of triad. J. Immunol. 194, 5375–5387, https://doi.org/10.4049/jimmunol.1401909
10 Prescott, N.J., Lehne, B., Stone, K., Lee, J.C., Taylor, K., Knight, J. et al. (2015) UK IBD Genetics Consortium. Pooled Sequencing of 531 genes in inflammatory bowel disease identifies an associated rare variant in BTNL2 and implicates other immune related genes. PLoS Genet. 11, e1004955, https://doi.org/10.1371/journal.pgen.1004955
11 Man, C.L., Mu, W.T., Chang, Y. and Zhao, D.X. (2015) Akirin2 expression in response to vaccine- induced immunity in chicken. Genet. Mol. Res. 14, 17489–17495
12 Berger, T., Saunders, M.E. and Mak, T.W. (2013) Dissection of signaling in inflammation: three novel inflammatory regulators. Cold Spring Harb. Symp. Quant. Biol. 78, 141–147, https://doi.org/10.1101/sqb.78.020107
13 Wang, H., Be, L., Shah C, A., Horvath, E. and Eklund, E.A. (2011) A protein ubiquitination by activating transcriptional of ARIH2, the gene encoding Triad1. J. Biol. Chem. 286, 16832–16845, https://doi.org/10.1074/jbc.M110.213975
14 Shimizu, Y., Taraborelli, L. and Walczak, H. (2015) Linear ubiquitination in immunity. Immunol. Rev. 266, 190–207, https://doi.org/10.1111/imr.12309
15 Zhang, M., Zhu, X., Zhang, Y., Chen, J., Sivaprakasam, S., Gurav, A. et al. (2015) RCAD/Ufl1, a Ufm1 E3 ligase, is essential for hematopoietic stem cell function and murine hematopoiesis. Cell Death Differ. 22, 1922–1934, https://doi.org/10.1038/cdd.2015.51
16 Kathania, M., Khare, P., Zeng, M., Cantarel, B., Zhang, H., Ueno, H. et al. (2016) Itch inhibits IL-17-mediated colon inflammation and tumorigenesis by RORγt ubiquitination. Nat. Immunol. 17, 997–1004, https://doi.org/10.1038/ni.3488
17 Amina, M., Sara, O., Stefania, C., Cantarel, B., Zhang, H., Ueno, H. et al. (2018) The environmental sensor AHR protects from inflammatory damage by maintaining intestinal stem cell homeostasis and barrier integrity. *Immunity* **49**, 353–362, [https://doi.org/10.1016/j.immuni.2018.07.010](https://doi.org/10.1016/j.immuni.2018.07.010)

18 Yang, Q., Bai, S.Y., Li, L.F., Li, S., Zhang, Y., Munir, M. et al. (2019) Human hemoglobin subunit beta functions as a pleiotropic regulator of the RIG-I/MDA5-mediated antiviral innate immune responses. *J. Virol.* **93**, e00718–e00719, [https://doi.org/10.1128/JVI.00718-19](https://doi.org/10.1128/JVI.00718-19)

19 Mhawech, P., Berczy, M., Assaly, M. et al. (2004) Human achaete-scute homologue (hASH1) mRNA level as a diagnostic marker to distinguish esthesioneuroblastoma from poorly differentiated tumors arising in the sinonasal tract. *Am. J. Clin. Pathol.* **122**, 100–105, [https://doi.org/10.1309/QD0K9Q1JHB6B5G0Q](https://doi.org/10.1309/QD0K9Q1JHB6B5G0Q)