Ubiquitin-specific peptidase 18 regulates the differentiation and function of Treg cells

Lu Yang a, Yukai Jing a, Danqing Kang a, Panpan Jiang a, Na Li b, Xinrong Zhou c, Yan Chen d, Lisa S. Westerberg e, Chaohong Liu a,∗

a Department of Pathogen Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, 430030, PR China
b Department of Immunology, School of Medicine, Yangtze University, Jingzhou, Hubei Province, 434023, PR China
c Department of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, 430030, PR China
d The Second Department of Pediatrics, Affiliated Hospital of Zunyi Medical University, Zunyi, GuiZhou Province, 563000, PR China
e Department of Microbiology Tumor and Cell Biology, Karolinska Institutet, Stockholm, 17177, Sweden

Received 8 January 2020; received in revised form 2 March 2020; accepted 11 March 2020
Available online 19 March 2020

Abstract Ubiquitin-specific peptidase 18 (USP18) plays an important role in the development of CD11b+ dendritic cells (DCs) and Th17 cells, however, its role in the differentiation of other T cell subsets, especially in regulatory T (Treg) cells, is unknown. In our study, we used Usp18 KO mice to study the loss of USP18 on the impact of Treg cell differentiation and function. We found that USP18 deficiency upregulates the differentiation of Treg cells, which may lead to disrupted homeostasis of peripheral T cells, and downregulates INF-γ, IL-2, IL-17A producing CD4+ T cells and INF-γ producing CD8+ T cells. Mechanistically, we also found that the upregulation of Tregs is due to elevated expression of CD25 in Usp18 KO mice. Finally, we found that the suppressive function of Usp18 KO Tregs is downregulated. Altogether, our study was the first to identify the role of USP18 in Tregs differentiation and its suppressive function, which may provide a new reference for the treatment of Treg function in many autoimmune diseases, and USP18 can be used as a new therapeutic target for precise medical treatment.

Copyright © 2020, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
**Introduction**

Ubiquitin-specific peptidase 18 (USP18) removes ubiquitin from its substrates. The Usp18 is a 43-kDa protein that is homologous to ubiquitin-specific proteases (UBPs) and is therefore also known as Ubp43. It was first cloned from mice expressing the acute myelogenous Leukemia fusion protein AML1-ETO by Liu et al. USP18 is expressed in several tissues with different levels. For instance, USP18 is highly expressed in liver, spleen and thymus, but clearly detectable in lung tissue, adipose tissue and bone marrow. USP18 is also expressed in lymphocytes and hematopoietic cells, including splenic B and T cells. In T cells, USP18 is highly expressed in naive or memory, as well as highly maintained in Th0, Th1, Th17 cells and natural regulatory T cells but decreased in Th2 cells and inducible regulatory T cells. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

As a critical subset of T cells, regulatory T cells (Tregs) mediate peripheral tolerance and maintain the immune homeostasis. In the early stage, the concept of ‘suppressor’ T cells has been arised. Next, Sakaguchi identified a unique CD4+CD25+ T population and named it regulatory T cells [Tregs]. Tregs are classified into natural Tregs and inducible regulatory T cells. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

As a critical subset of T cells, regulatory T cells (Tregs) mediate peripheral tolerance and maintain the immune homeostasis. In the early stage, the concept of ‘suppressor’ T cells has been arised. Next, Sakaguchi identified a unique CD4+CD25+ T population and named it regulatory T cells [Tregs]. Tregs are classified into natural Tregs and inducible regulatory T cells. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

Inducible co-stimulator (ICOS, CD278) is a member of the CD28 family of costimulatory molecules, and is lowly expressed in resting naive T cells but is rapidly upregulated after activation by TCR ligation and CD28. ICOS is expressed in unpolarized CD4+ T cells, activated Th1, Th2, Th17, Thf and Treg lineages. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

As a critical subset of T cells, regulatory T cells (Tregs) mediate peripheral tolerance and maintain the immune homeostasis. In the early stage, the concept of ‘suppressor’ T cells has been arised. Next, Sakaguchi identified a unique CD4+CD25+ T population and named it regulatory T cells [Tregs]. Tregs are classified into natural Tregs and inducible regulatory T cells. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

As a critical subset of T cells, regulatory T cells (Tregs) mediate peripheral tolerance and maintain the immune homeostasis. In the early stage, the concept of ‘suppressor’ T cells has been arised. Next, Sakaguchi identified a unique CD4+CD25+ T population and named it regulatory T cells [Tregs]. Tregs are classified into natural Tregs and inducible regulatory T cells. USP18 is a negative regulator of type I and type III interferon signaling, which plays an essential role in the innate antiviral response. What’s more, USP18 was found involved in the development of DCs and Th17 cells. However, in the differentiation of other T cell subsets remains to be explored.

**Materials and methods**

**Mice**

Usp18 KO mice on the C57 BL/6 background were donated by Prof. Bo Zhong’s lab in Wuhan University of China as described before. 8-Week-old Usp18 KO mice and sex-matched littersmates were used in all experiments. All mice were fed and housed in a specific pathogen-free condition and all experiments were performed according to the protocols from the Chinese Council on Animal Care and approved by the Ethics Committee of Animal Experimentation of Tongji Medical College (Wuhan, China).

**Cells preparation**

Spleens, thymuses and LNs from Usp18 KO and WT mice were harvested and mashed into cell suspensions in DMEM containing 2% FBS, and RBCs in splenocytes were lysed with ACK (TIANGEN, RT122-01).

**Flow cytometry analysis of cell surface molecule and intracellular molecule**

Cell suspensions were incubated with anti-mouse CD16/ CD32 (Biolegend, 101,319). For cell surface flow cytometry, cells from spleens, thymuses and LNs were stained with specific antibodies for surface antigens as follows: PE-anti-CD4 (Biolegend, 100,408), Pacific Blue-anti-CD4 (Biolegend, 100,531), Brilliant Violet 510-anti-CD8a (Biolegend, 102,012), PerCP/Cy5.5-anti-CD44 (Biolegend, 103,032), PE/Cy7-anti-CD279 (PD-1) (Biolegend, 145,206), PE/Cy7-anti-CD62L (Biolegend, 104,418), PE-anti-CD278 (ICOS) (Biolegend, 107,705), APC-anti-CD304 (Neutrophil-1, Nrp1) (Biolegend, 145,206), PE/Cy7-anti-CD279 (PD-1) (Biolegend, 109,110). For intracellular staining, cells were fixed and permeabilized with Fixation/Permeabilization Kit (eBioscience, 00–5123, 00–5223), washed with Permeabilization Buffer (eBioscience, 00–8333) and stained with PE-Cy7-anti-ki67 (eBioscience, 25-5698-82), PE-anti-CTLA4 (Biolegend, 106,306), AF488-anti-Foxp3 (Thermo Scientific, 53-5773-82), PE-anti-Foxp3 (Biolegend, 126,404). For apoptosis analysis, cells were stained with FITC-AnnexinV (Biolegend, 460,906) in AnnexinV Binding Buffer (Biolegend, 422,201). Samples were analyzed by LSRII multicolor flow cytometer (BD Biosciences CA, USA).
and data analysis was performed using the FlowJo software (Tree Star, USA).

T cell stimulation and intracellular cytokine staining

2 × 10⁶ lymphocytes from spleens, thymuses and LNs were plated in round-bottomed 96-well plates in 1 ml RPMI complete media containing PMA (50 ng/ml, Sigma, P1585-1 MG), GolgiStop (1:1000, BD Biosciences, 554,724) and ionomycin (1 µM, CST, 99955). After culturing for 5 h in 37 °C, 5% CO₂, cells were collected and stained with PE-anti-CD4 (Biolegend, 100,408), Brilliant Violet 510-anti-CD8α (Biolegend, 100,751), PerCP/Cy5.5-anti-CD44 (Biolegend, 103,032), APC/Cy7-anti-TCRβ (Biolegend, 109,220). Then cells were fixed, permeabilized and stained with APC-anti-IL-4 (Biolegend, 504,106), PE/CY7-anti-CD25 (Biolegend, 100,412). Samples were then analyzed by flow cytometer (BD Biosciences CA, USA) and data analysis was performed with FlowJo software (Tree Star, USA).

Cell sorting and Treg suppression assay

Splenic CD4⁺ naïve T cells and Treg cells were sorted by Naïve CD4⁺ T Cell Isolation Kit (Miltenyi, 130-104-453) and CD4⁺ CD25⁻ Reg T Cell Isolation Kit (Miltenyi, 130-091-041). CD4⁺ naïve T cells were labeled with CellTrace Violet (Thermo Scientific™, C34557, C34571) at a concentration of 5 µM for 10 min at 37 °C, followed by incubation with Treg cells at a different ratio in a U-bottom 96-well plate that pretreated with anti-CD3/CD28 antibodies (Biox Cell, BE0015). After 72 h, cells were collected to stain with 7-AAD and APC-anti-CD4 antibody (Biolegend, 504,106), PE/CY7-anti-IFN-γ (Biolegend, 505,826) and Brilliant Violet 421-anti-IL-17A (Biolegend, 506,925).

Bone marrow chimera generation

To generate mixed bone marrow (BM) chimeras, CD45.1 recipient mice were pre-treated drinking water with gentamycin (Biofrax, 1453GR005, 480,000 U/L) and erythromycin (Biofresh, 375,000 U/L) for one week, and then were irradiated (7 Gy) 4 h prior to BM cell transfer. BM cells (2.5 × 10⁶) were obtained from WT or Usp18 KO mice and mixed with CD45.1 congenic BM cells (1:1) and were injected i.v. into irradiated recipient mice. After transfer, recipient mice continued to receive antibiotic-containing drinking water for four weeks and were analyzed eight weeks later.

Statistical analysis

Two-paired Student’s t tests were carried out with Prism GraphPad Prism 6 Software (San Diego, CA) to assess the statistical significance. The difference was considered significant when *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns, no significance.

Results

USP18 deficiency alters the homeostasis of peripheral T cells, but not the development of T cells in thymus

To analyze the physiological role of USP18 in T cells, we first analyzed thymic development of CD4⁺ and CD8⁺ T cells, and no significant change of the frequency was observed between Usp18 KO and WT mice (Fig. 1A–C). Meanwhile, we did not find significant difference in the absolute numbers of CD4⁺ CD8⁻ (SP4) and CD4⁺ CD8⁺ (SP8) thymocytes in Usp18 KO and WT mice (Fig. 1B and C). Next, we analyzed the T cell homeostasis in the periphery, and the frequencies as well as the total numbers of CD4⁺ and CD8⁺ T cells in spleen and lymph nodes (LNs) were unaltered between Usp18 KO and WT mice (Fig. 1A–C). However, we observed a higher frequency of naïve CD4⁺ T cells in spleen and naïve CD8⁻ (CD44⁺CD62li) T cells in spleen and LNs in Usp18 KO mice than that in WT mice (Fig. 1D–G). On the contrary, the frequencies of activated effector CD4⁺ in spleen and activated effector CD8⁻ (CD44hiCD62li) T cells in spleen and LNs were reduced in Usp18 KO mice than that in WT mice (Fig. 1D–G). Accordingly, these results indicated that USP18 is critical for the homeostasis of peripheral T cells but not for the thymocyte development.

USP18 deficiency upregulates the differentiation of Treg cells

Because regulatory T (Treg) cells play an essential role in maintaining the naïve T cell pool, we further investigated the effect of USP18 deficiency on Treg cells. We found that the percentage of CD4⁺ CD25⁺ Treg cells was significantly increased in spleen, LN and thymus of Usp18 KO mice compared with that of WT mice (Fig. 2A and C). Similarly, the frequency of CD4⁺ Foxp3⁺ Treg was also increased in spleen and LN except for the thymus of Usp18 KO mice (Fig. 2B and D). What’s more, the change of cell number of CD4⁺ CD25⁺ or CD4⁺ Foxp3⁺ was in keeping with that of percentage (Fig. 2C and D). Furthermore, we tested the expression of CD25 and Foxp3 by flow cytometry. Higher level of CD25 was found in spleen, LN and thymus of Usp18 KO mice compared with that of WT mice (Fig. 2A and C). Similarly, the frequency of CD4⁺ Foxp3⁺ Treg was also increased in spleen and LN except for the thymus of Usp18 KO mice (Fig. 2B and D). Moreover, we tested the expression of Foxp3 by flow cytometry. Higher level of CD25 and Foxp3 by flow cytometry. Higher level of CD25 was found in spleen, LN and thymus of Usp18 KO mice compared with that of WT mice (Fig. 2E and F). However, no significant difference in the Foxp3 expression was observed between Usp18 KO and WT mice (Fig. 2F and H). To further dissect the mechanism of the increase of Tregs, the apoptosis and proliferation of Treg were measured by Annexin V and Ki-67 staining respectively. No obvious difference was found in the percentage of Annexin V⁻ and Ki-67⁻ of Treg as well as the expression of Annexin V and Ki-67 (Fig. 2I–L). Together, these findings identified USP18 deficiency up-regulates the generation of Tregs both in central and peripheral tissues.

To further determine whether USP18 affects the development of Treg is intrinsic, we generated mixed bone marrow chimera mice with WT and Usp18 KO mice to remove the secondary impact on KO Treg cells from environment. Interestingly, analyses of CD45.1⁻ T cells showed that the percentage of CD4⁺ CD25⁺ Treg cells was increased in spleen and LNs of Usp18 KO mice than that of WT mice,
Figure 1  USP18 deficiency alters the homeostasis of peripheral T cells, but not the development of T cells in thymus. Flow cytometry analyzing the expression of CD4 and CD8 in lymphocytes of thymus, spleen and LN derived from Usp18 KO (n = 8) and WT (n = 8) mice. Shown are representative dot plots (A) as well as percentages and absolute numbers of CD4+ (B) and CD8+ T cells (C). Expression of CD44 and CD62L on CD4+ TCR+ or CD8+ TCR+ T cells of spleen and LN derived from Usp18 KO (n = 6) and WT (n = 6) mice was analyzed by flow cytometry. Shown are representative dot plots and percentages of CD62LhiCD44lo naive CD4+ T cells and CD62LloCD44hi activated CD4+ T cells (D–E). Dot plots and percentages of naive and activated CD8+ T cells are shown in (F–G). Data are representative of three independent experiments and values are expressed as mean ± SD. *P < 0.05, **P < 0.01, ns, not significant.
Figure 2  USP18 deficiency upregulates the differentiation of Treg cells. Representative figures shown the expression of CD25 in CD4⁺ T cells from the spleen, thymus and LN of WT and Usp18 KO mice (A) Percentages and absolute numbers of CD4⁺CD25⁺ T cells from the spleen, thymus and LN of WT (n = 6) and KO littermates (n = 6) (C) Representative figures, percentages and absolute numbers of CD4⁺Foxp3⁺ T cells from the spleen, thymus and LN of WT (n = 6) and KO littermates (n = 6) as indicated in (B–D) Flow cytometry analyzing expression of CD25 in CD4⁺CD25⁺ T cells (E–G) and Foxp3 in CD4⁺Foxp3⁺ T cells (F–H) from the spleen, thymus and LN of WT (n = 6) and Usp18 KO littermates (n = 6). Flow cytometry analyzing the apoptosis and proliferation of CD4⁺CD25⁺ and CD4⁺Foxp3⁺ T cells from the spleen, thymus and LN of WT (n = 4) and KO littermates (n = 4) (I–L). Shown are representative dot plots from one of three independent experiments. Flow cytometry analysis of CD25 and Foxp3 expression in CD45.2⁺CD4⁺ T cells of spleen, thymus and LN in mixed bone marrow chimeras (n = 5) eight weeks after transfer (M–O) *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns, not significant.
but no difference in the thymus (Fig. 2M and N). Similarly, the frequency of CD4\(^+\)Foxp3\(^+\) Treg cells in Usp18 KO mice were also higher in spleen and LNs, and equally in the thymus compared to WT mice (Fig. 2O). These observations collectively indicate an intrinsic role of USP18 in mediating the differentiation of Treg cells.

**USP18 deficiency decreases the ability of cytokine production by effector T cells**

Next, we detected the ability of effector T cells in Usp18 KO mice to produce cytokines. After stimulated with PMA, GolgiStop and ionomycin for 5 h, lymphocytes from spleens, LNs and thymus of Usp18 KO and WT mice were collected to detect intracellular cytokines with flow cytometry. We found that significantly decreased production of IFN-\(\gamma\) and IL-4 in CD4\(^+\) T cells of spleen and thymus from Usp18 KO mice compared with that of WT mice, but not for that of LN (Fig. 3A–D). The production of IL-17A in CD4\(^+\) T cells, reduced production of IFN-\(\gamma\) in spleen of Usp18 KO mice was found, but we didn’t found significant difference in thymus and LN (Fig. 3F and H). These results
Figure 4  USP18 deficiency downregulates the suppression function of Treg cells in vitro. Purified Treg cells of spleens from WT and Usp18 KO mice were cultured with CellTrace Violet-labeled naïve CD4\(^+\) T cells from WT mice and anti-CD3/CD28 antibodies at the indicated ratio for 72 h in vitro. The proliferation of naïve CD4\(^+\) T cells was measured by flow cytometry. Shown are representative images from at least two independent experiments (A). Flow cytometry analyzing the expression of ICOS (B–C), CTLA-4 (D–E), PD-1 (F–G) and neuropilin-1(Nrp1) (H–I) in CD4\(^+\)CD25\(^+\) and CD4\(^+\)Foxp3\(^+\) Treg cells from the spleen, thymus and LN of WT (n = 5) and KO littermates (n = 5). Shown are representative images and mean values (±SD) from three independent experiments. *P < 0.05; ns, not significant.
indicated that USP18 plays an essential role of maintaining the ability of cytokine production by effector T cells.

**USP18 deficiency downregulates the suppression function of Treg cells in vitro**

To identify whether USP18 is crucial for the function of Treg cells, we performed a suppression assay in vitro. Isolated and purified Treg cells of Usp18 KO or WT mice were co-cultured with naive CD4+ T cells from WT mice in the present of anti-CD3/CD28 for 72h, and then the proliferation of T naive cells was detected. We found that USP18-deficient Treg cells were less effective than WT Treg cells in inhibiting the proliferation of naive T cells in vitro (Fig. 4A). To further investigate the mechanism of how USP18 control the suppressive function of Treg cells, we detected several signature molecules in Treg cells related to suppressive function such as ICOS, CTLA-4, PD-1 and neuropilin-1(Nrp1). We found that the expression of ICOS in Usp18 KO Tregs in spleen was significantly increased compared with that of WT Tregs in spleen, but no obvious difference in thymus and LN (Fig. 4B and C). However, Tregs in Usp18 KO mice exhibited slightly decreased expression of CTLA-4 in thymus compared with that of WT mice, but not for that in spleen and LN (Fig. 4D and E). What’s more, we didn’t found any significant difference of PD-1 and neuropilin-1(Nrp1) in spleen, thymus and LN between Usp18 KO and WT mice (Fig. 4F–I). Accordingly, these findings suggested that USP18 is critical for the suppressive function of Tregs although the expression of ICOS is upregulated.

**Discussion**

So far, previous studies indicate that USP18 plays an essential role in the innate antiviral response and is involved in the development of DCs and Th17 cells, but there is no report to explore the role of USP18 in Tregs. In our study, we first use the Usp18 knock out animal model to investigate the effect of USP18 deficiency on Treg differentiation and suppressive function. Overall, USP18 is critical for the homeostasis of peripheral T cells and negatively regulates the differentiation of Tregs by downregulating the expression of CD25. What’s more, USP18 plays an essential positive role in maintaining the suppressive function of Tregs. However, using the germ-line deletion mice for Usp18, we cannot exclude the influence of other immune cells. In addition, it caused embryonic lethality that Usp18 KO mice were backcrossed to C57BL/6 mice to over five generations. So, our further study is aimed to explore the function of USP18 on Tregs by building the model of conditioned knockout mice through crossing Usp18<sup>flox/flox</sup> mice with Foxp3<sup>YFP-Cre</sup> mice and further validate our results in the bone marrow chimera mice model.

The transcription factor Foxp3, which cooperates with other transcription factors play an essential role in the development and function of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, can induce upregulation of CD25, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and the glucocorticoid-induced TNF receptor, and inhibit IL-4, IFN-γ, and IL-17 is produced by effector T cells. Previous researches have shown that the IL-2 signaling transmitted by high-affinity IL-2R is essential for the homeostasis of Treg cells, promotion of survival and immunosuppressive by inducing Foxp3 and CD25 expression. Our study illustrated the mechanism that USP18 deficiency upregulates the differentiation of Treg cells through upregulating the expression of CD25, and decreases the ability of cytokine production by effector T cells. Besides, the result that decreased percentage of producing-IL-17 cells in Usp18 KO mice is also in accordance with the finding before. We found that changes in CD25 expression were inconsistent with changes in Foxp3, which may be caused by the deubiquitination of Foxp3 by USP18, similarly to the effect of USP21 on Foxp3. It is worthy of figuring out the defined mechanisms.

Strangely, but interestingly, we found that USP18 deficiency downregulates the suppressive function of Tregs, whereas the expression of ICOS is upregulated, which may be caused by the inflammatory stimulation in the microenvironment. In addition, the influence of the increased percentage of Tregs in Usp18 KO mice maybe outweighs that of downregulated suppressive function. Further investigation is also needed.

In summary, we first report here an important role of USP18 in regulatory T cell differentiation and function. These results are helpful for understanding and treatment of Treg function associated diseases. Treg dysfunction exists in all kinds of autoimmune diseases, and USP18 could be used as a new therapeutic target for precise medical treatment.

**Authors contribution**

L. Yang drafted the initial manuscript. C. Liu designed the study, reviewed and revised the manuscript. L. Yang, D. Kang, P. Jiang and Na. Li performed the flow cytometry assay. L. Yang and Y. Jing carried out the cell sorting and Treg suppression assay. L. Yang analyzed the data and generated figures. X. Zhou, Y. Chen and L. Westerberg assisted with the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

**Conflict of Interests**

The authors have no financial conflict of interest.

**Acknowledgements**

This work was supported by National Natural Science Foundation of China (grant numbers 81861138002, 81722002, 31970839, 31900654).

**Abbreviations**

- **USP18** Ubiquitin-specific peptidase 18
- **DC** Dendritic cells
- **Treg** Regulatory T cell
- **KO** knockout
- **UBPs** ubiquitin-specific proteases
- **PMA** Phorbol 12-myristate 13-acetate
- **ICOS** inducible costimulatory
- **TGF-β** transforming growth factor-β
 CTLA-4 cytotoxic T lymphocyte-associated antigen 4  
Nrp1 neuropilin-1  
PD-1 programmed cell death protein 1  
LN lymph node  

References

1. Liu LQ, Ilaria Jr R, Kingsley PD, et al. A novel ubiquitin-specific protease, UBP43, cloned from leukemia fusion protein AML1-ETO-expressing mice, functions in hematopoietic cell differentiation. *Mol Cell Biol*. 1999;19(4):3029–3038.

2. Schwer H, Liu LQ, Zhou L, et al. Usp18-deficient mammary epithelial cells create an antitumour environment driven by hypersensitivity to IFN-lambda and elevated secretion of Cxcl10. *EMBO Mol Med*. 2013;5(7):1035–1050.

3. Francis-Newton V, Magno de Freitas Almeida G, Payelle-Brogard B, et al. USP18-based negative feedback control is induced by type I and type III interferons and specifically inactivates interferon alpha response. *PloS One*. 2011;6(7), e22200.

4. Burkart C, Arimoto K, Tang T, et al. Usp18 deficient mammary epithelial cells create an antitumour environment driven by hypersensitivity to IFN-lambda and elevated secretion of Cxcl10. *EMBO Mol Med*. 2013;5(7):1035–1050.

5. Cong XL, Lo MC, Reuter BA, Yan M, Fan JB, Zhang DE. Usp18 promotes conventional CD11b+ dendritic cell development. *J Immunol*. 2012;188(10):4776–4781.

6. Gershon RK. A disquisition on suppressor T cells. *Transplant Rev*. 1975;26:170–185.

7. Gershon RK, Cohen P, Hencin R, Liebhaver SA. Suppressor T cells. *J Immunol*. 1972;108(3):586–590.

8. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immuno-logic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). *J Immunol*. 1995;155(3):1115–1124.

9. Hulotto A, Dittrich AM, Beier KC, et al. ICOS is an inducible T cell co-stimulator structurally and functionally related to CD28. *Nature*. 1999;397(6716):263–266.

10. McAdam AJ, Chang TT, Lumelsky AE, et al. Mouse inducible costimulatory molecule (ICOS) expression is enhanced by CD28 costimulation and regulates differentiation of CD4+ T cells. *J Immunol*. 2000;165(9):5035–5040.

11. Tan AH, Goh SY, Wong SC, Lam KP. T helper cell-specific regulation of inducible costimulator expression via distinct mechanisms mediated by T-bet and GATA-3. *J Biol Chem*. 2008;283(1):128–136.

12. Nakae S, Iwakura Y, Suto H, Galli SJ. Phenotypic differences between Th1 and Th17 cells and negative regulation of Th1 cell differentiation by IL-17. *J Leukoc Biol*. 2007;81(5):1258–1268.

13. Akiba H, Takeda K, Kojima Y, et al. The role of ICOS in the CXCR5+ follicular B helper T cell maintenance in vivo. *J Immunol*. 2005;175(4):2340–2348.

14. Burmeister Y, Lischke T, Dahler AC, et al. ICOS controls the pool size of effector-memory and regulatory T cells. *J Immunol*. 2008;180(2):774–782.

15. Ito T, Hanabuchi S, Wang YH, et al. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. *Immunity*. 2008;28(6):870–880.

16. Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science*. 1976;193(4257):1007–1008.

17. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat Immunol*. 2005;6(11):1142–1151.

18. D’Cruz LM, Klein L. Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. *Nat Immunol*. 2005;6(11):1152–1159.

19. Setoguchi R, Hori S, Takahashi T, Sakaguchi S. Homeostatic maintenance of natural Foxp3+ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J Exp Med*. 2005;201(5):723–735.

20. Malek TR, Yu A, Vincen K, Scibelli P, Kong L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2beta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity*. 2002;17(2):167–178.

21. Suzuki H, Kundig TM, Furlonger C, et al. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science*. 1995;268(5216):1472–1476.

22. Ritchie KJ, Malakhov MP, Hetherington CJ, et al. Dysregulation of protein modification by ISG15 results in brain cell injury. *Genes Dev*. 2002;16(17):2207–2212.

23. Kim KI, Malakhova OA, Hoebe K, Yan M, Beutler B, Zhang DE. Enhanced antibacterial potential in UBP43-deficient mice against Salmonella typhimurium infection by up-regulating type I IFN signaling. *J Immunol*. 2005;175(2):847–854.

24. Malek TR. The biology of interleukin-2. *Annu Rev Immunol*. 2008;26:453–479.

25. Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol*. 2005;6(4):331–337.

26. Li Y, Lu Y, Wang S, et al. USP21 prevents the generation of T-helper-1-like Treg cells. *Nat Commun*. 2016;7, e13559.