In Vitro Study on the Synergistic Regulation of CD8⁺T Cell Subset Differentiation and Effector Function by IL-33 and Eomes Deficiency

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Abstract

Objective This study aims to investigate the synergistic regulatory effect of IL-33 and Eomes transcription factor deficiency on the differentiation and effector function of $CD8^+$ T memory cell subsets.

Methods Using wild-type (WT) and Eomes knockout (EKO) mouse models, combined with flow cytometry analysis, we systematically evaluated the phenotypic and functional changes of $CD8^+$ T cells under different culture conditions in vitro.

Results TWS119 upregulated the expression of stem cell-like memory cells (TSCM) when Eomes was deficient. Eomes deficiency downregulated the expression of Ly108 and upregulated the expression of IFN-γ. The combination of IL-33 and Eomes deficiency upregulated the expression of effector-like cells and downregulated the expression of exhausted-like cells, promoting the differentiation of $CD8^+$ T cells towards the effector direction.

Conclusion There is a synergistic mechanism between IL-33 and Eomes in regulating the differentiation of $CD8^+$ T cells, providing a new theoretical basis for optimizing T cell immunotherapy strategies.

1. Introduction

The process of $CD8^+$ T cell differentiation into subsets such as effector cells, effector memory cells, and stem cell-like memory cells is regulated by multiple cytokines and transcription factors, among which IL-33 and Eomes factors play key roles in immune responses.

IL-2 promotes the clonal expansion and effector differentiation of $CD8^+$ T cells by activating the JAK-STAT5 signaling pathway[1]; IL-12 combined with PD-1 inhibitors can synergistically enhance the antitumor effect[2]; IL-7/IL-15 maintain the homeostasis of memory T cells by regulating metabolic pathways such as fatty acid oxidation and glycolysis[3]. In addition, the GSK-3β inhibitor TWS119 promotes the differentiation and maintenance of stem cell-like memory T cells (TSCM) by activating the Wnt/β-catenin signal[4].

IL-33, an alarmin of the IL-1 family, activates the NF-κB and MAPK signaling pathways by binding to the ST2 receptor on the surface of target cells, playing a key role in infection or tissue damage[5]. Studies have shown that IL-33 can significantly enhance the activation and cytotoxic function of $CD8^+$ T cells, promote the expression of effector molecules such as granzyme B, and thus improve the killing efficiency of infected cells or tumor cells[6]. In addition, IL-33 is crucial for the formation of memory T cells: in a chronic infection model, IL-33 promotes the expansion of $Tcf\text{-}1^+$ $CD8^+$ T cells and maintains their stem cell-like characteristics, endowing cells with the ability to self-renew and differentiate into multiple effector subsets, laying a foundation for long-term immune protection[7].

Eomesodermin (Eomes) is a core transcription factor for maintaining the function of memory T cells, regulating gene expression by binding to specific DNA sequences. On the one hand, Eomes upregulates the expression of cytotoxic proteins such as Granzyme B, directly enhancing the killing ability of $CD8^+$ T cells[8]; on the other hand, it prolongs the survival time of memory T cells by promoting the synthesis of anti-apoptotic proteins such as Bcl-2, ensuring the continuity of immune surveillance[9]. In differentiation regulation, the level of Eomes differentially regulates the balance between effector and memory T cells: high expression drives effector T cell differentiation, while moderate expression maintains the stemness and rapid reactivation potential of memory T cells[10]. The latest research suggests that Eomes may stabilize the characteristics of memory T cells through epigenetic modification, providing a new target for adoptive cell therapy.

The two factors, from the perspectives of cytokine signaling and transcriptional regulation, precisely regulate the activation, differentiation, and memory maintenance of $CD8^+$ T cells, providing important targets for anti-infection, anti-tumor immunotherapy, and adoptive cell therapy. The promotion of memory T cell stemness by IL-33 and the regulation of the effector-memory balance by Eomes are expected to become new directions for optimizing immune therapy strategies.

2. Materials and Methods

2.1 Materials

This study used WT wild-type mice and Eomes-deficient EKO mice. We euthanized the mice by CO_2asphyxiation and Our animal ethics approval number is: SUDA20250616A09. We confirm that all methods were carried out in accordance with relevant guidelines and regulations and all methods are reported in accordance with ARRIVE guidelines.all experimental protocols were approved by Animal Ethics Committee of Soochow University. The wild-type mice were bought from JiHui and EKO mice were brought by Zhu.he reagents used included plate-bound anti-CD3, plate-bound anti-CD28, IL-2, IL-7, IL-12, IL-15, IL-33, and TWS119.

2.2 Methods

2.2.1 Preparation of Single-Cell Suspensions from Mouse Spleens

1) Take 6-8-week-old WT and EKO mice, sacrifice them and soak them in 75% ethanol solution. Prepare sterilized scissors and forceps, take the mouse spleen tissue in a sterile culture dish pre-added with 5-6 mL of PBS containing 1% FBS;

2) Gently grind the spleen tissue with the frosted surface of a sterile glass slide in the same direction, filter it through a sterile 200-mesh filter membrane, collect the cell suspension into a 50 mL centrifuge tube, centrifuge at 1200 rpm, 4°C for 5 min, discard the supernatant, and gently resuspend the cells at the bottom of the centrifuge tube;

3) Add 5 mL of 1×RBC Lysis Buffer to the centrifuge tube, mix gently to ensure complete lysis, place on ice for 5 min to lyse red blood cells, then add 25 mL of PBS containing 1% FBS to terminate cell lysis, centrifuge at 1200 rpm, 4°C for 5 min, discard the supernatant, and gently resuspend the cells at the bottom of the centrifuge tube;

4) Add 1 mL of PBS containing 1% FBS to resuspend the cells and count them under a microscope, then place them on ice for later use.

2.2.2 Immunomagnetic Bead Enrichment of Mouse Spleen-Derived CD8⁺ T Cells

1) Resuspend cells at a concentration of 1×10^7 cells/100 mL, add 10 μL of mouse CD8α MicroBeads to every 10^7 cells resuspended in 90 μL of PBS containing 1% FBS, mix gently, and incubate at 4°C in the dark for 15 min; 2) Add 8 mL of PBS containing 1% FBS to wash the cells, centrifuge at 1200 rpm, 4°C for 5 min, discard the supernatant, gently resuspend the cells at the bottom of the centrifuge tube, and add 3 mL of PBS containing 1% FBS to obtain a cell suspension;

2) Wash the sorting column placed on the magnetic rack twice with 2 mL of PBS containing 1% FBS each time, slowly add the spleen cell suspension obtained in the previous step to the sorting column, avoiding bubbles during the whole process, and then wash the sorting column three times with 2 mL of PBS containing 1% FBS each time;

3) Remove the sorting column, add 5 mL of PBS containing 1% FBS, quickly push out the liquid and collect it into a 15 mL centrifuge tube, then wash the sorting column once with 3 mL of PBS containing 1% FBS, quickly and forcefully push out the liquid to obtain the CD8⁺ T cell suspension, count under a microscope and determine cell purity by flow cytometry, and store on ice for later use.

2.2.3 In Vitro Induction and Culture of Purified Mouse Spleen-Derived CD8⁺ T Cells

1) Take a flat-bottom 48-well plate, add 200 μL of DPBS containing anti-mouse purified CD3ε (1.25 μg/mL) and anti-mouse purified CD28 (1.25 μg/mL) to each well, and coat overnight at 4°C;

2) Aspirate the coating solution with a vacuum pump, and wash 1-2 times with 200 μL/well of DPBS;

3) Adjust the density of the sorted spleen-derived CD8⁺ T cells to 6×10^5/500 μL/well with RPMI 1640 complete medium containing 10% FBS, and add the following cytokines to each group:

+TWS119：IL-2 (20 U/mL) + TWS119 (2 μM) + IL-7 (10 ng/mL) + IL-15 (10 ng/mL);

Blank Group: IL-2 (20 U/mL); -

Place in a 37°C, 5% CO_2 incubator for culture.

4) After 48 h, transfer the cells in the plate to a new 48-well plate without anti-mouse purified CD3ε and anti-mouse purified CD28 coating, and supplement with the corresponding group of cytokines IL-2 (20

U/mL) + IL-12 (3.4 ng/mL), and place in a 37°C, 5% CO_2 incubator for culture;

5) After 24 h, add the following cytokines to each group according to the grouping:

+IL-33: IL-2 (20 U/mL) + IL-33 (30 ng/mL);

Blank group: IL-2 (20 U/mL);

Place in a 37°C, 5% CO_2 incubator for culture.

2.2.4 Flow Cytometry Detection of Related Molecule Expression

1) Collect cells from each group at 6 h and 24 h, wash with PBS containing 1% FBS, and centrifuge at 3000 g, 4°C for 3 min;

2) Add antibodies diluted at the following ratios to the samples: CD45-Percpcy5.5 (1:200), CD8-PB450 (1:160), PD-1-PECF594 (1:100), CD44-BV785 (1:200), CD62L-A750 (1:200), Ly108-APC (1:250), Eomes-Pecy7 (1:100), IFN-γ-FITC (1:100), incubate at 4°C in the dark for 30 min;

3) Wash with PBS containing 1% FBS, centrifuge at 3000 g, 4°C for 3 min, take the precipitate, and perform flow cytometry detection.

3. Results

3.1 TWS119 Upregulates the Expression of $Ly108^+CD62L^+CD8^+$ Stem Cell-Like Memory Cells (TSCM) in the Absence of Eomes

To accurately evaluate the effect of Eomes deficiency and TWS119 on TSCM, the proportions of TSCM in the WT group, EKO group, WT+TWS119 group, and EKO+TWS119 group were compared.

As shown in Figure 1, at 6 h, the TSCM proportions in the WT group and EKO group were 39.8% and 29.0%, respectively, a decrease of 10.8%; at 24 h, the TSCM proportions in the WT group and EKO group were 22.3% and 13.9%, respectively, a decrease of 8.4%. This indicates that Eomes deficiency can downregulate the expression of TSCM. At 6 h, the TSCM proportions in the WT group and WT+TWS119 group were 39.8% and 41.1%, respectively, an increase of 1.3%; at 24 h, the TSCM proportion in the WT group and EKO+TWS119 group were 22.3% and 21.1%, respectively, a decrease of 1.2%. This shows that the role of single TWS119 in the expression of TSCM is unstable. At 6 h, the TSCM proportions in the EKO group and EKO+TWS119 group were 29.0% and 36.2%, respectively, an increase of 7.2%; at 24 h, the TSCM proportions in the EKO group and EKO+TWS119 group were 13.9% and 14.7%, respectively, an increase of 0.8%. This indicates that in the case of Eomes deficiency, the presence of TWS119 can partially rescue the downward trend of TSCM.

In general, in the absence of Eomes, TWS119 can upregulate the expression of TSCM, but this upregulation is not sufficient to offset the downregulation caused by Eomes deficiency.

3.2 Eomes Deficiency Downregulates the Expression of Ly108 and Upregulates the Expression of IFN-γ

To explore the effect of Eomes deficiency on Ly108 expression, the proportion of $Ly108^+$ cells in the WT group and EKO group was compared, as shown in Figure 2(A). At 6 h, the proportions in the WT group and EKO group were 81.1% and 70.9%, respectively, a significant decrease of 10.3% ($p<0.005$); at 24 h, the proportions in the WT group and EKO group were 54.3% and 40.8%, respectively, a significant decrease of 14.5% ($p<0.005$). The data at the two time points indicate that Eomes deficiency significantly downregulates the expression of Ly108.

To explore the effect of Eomes deficiency on IFN-γ expression, the proportion of $IFN\text{-}\gamma^+$ cells in the WT group and EKO group was compared, as shown in Figure 2(B). At 6 h, the proportions in the WT group and EKO_CON. group were 9.30% and 14.4%, respectively, a significant increase of 5.1% ($p<0.001$); at 24 h, the proportions in the WT group and EKO group were 8.81% and 13.5%, respectively, a significant increase of 4.69% ($p<0.001$). The data at the two time points indicate that Eomes deficiency significantly upregulates the expression of IFN-γ.

In general, Eomes deficiency can downregulate the expression of Ly108 and upregulate the expression of IFN-γ.

3.3 IL-33 Upregulates the Expression of $Ly108^-IFN\text{-}\gamma^+CD8^+$ T Cells (TEFF)

To accurately evaluate the effect of Eomes deficiency and TWS119 on effector-like subset cells, the TEFF proportion gap between the WT group and WT+IL-33 group was compared.

As shown in Figure 3, at 6 h, the TEFF proportions in the WT group and WT+IL-33 group were 7.49% and 12.0%, respectively, a significant increase of 4.51% ($p<0.005$); at 24 h, the TEFF proportions in the WT group and WT+IL-33 group were 25.2% and 36.0%, respectively, an increase of 10.8%. This indicates that the role of IL-33 can upregulate the expression of TEFF.

3.4 IL-33 Synergizes with Eomes Deficiency to Downregulate the Expression of $Ly108^+PD\text{-}1^+CD8^+$ Exhausted Precursor Cells (Tpex)

To accurately evaluate the effect of Eomes deficiency and TWS119 on Tpex, the proportion of exhausted-like subsets in the WT group, WT+IL-33 group, EKO group, and EKO+IL-33 group was compared.

As shown in Figure 4, at 6 h, the proportions of exhausted-like subsets in the WT group and EKO group were 23.1% and 18.5%, respectively, a significant decrease of 4.6% ($p<0.005$); at 24 h, the proportions in the WT group and EKO group were 1.15% and 1.11%, respectively, a decrease of 0.04%. This indicates that Eomes deficiency can downregulate the proportion of Tpex. At 6 h, the Tpex proportions in the WT group and WT+IL-33 group were 23.1% and 23.3%, respectively, an increase of 0.2%; at 24 h, the Tpex proportions in the WT group and WT+IL-33 group were 1.15% and 1.55%, respectively, an increase of 0.4%. This shows that the role of single IL-33 on Tpex is a promoting effect. At 6 h, the Tpex proportions in the EKO group and EKO+IL-33 group were 18.5% and 17.6%, respectively, a decrease of 0.9%; at 24 h,

the Tpex proportions in the EKO group and EKO+IL-33 group were 1.11% and 0.86%, respectively, a decrease of 0.25%. This indicates that in the case of Eomes deficiency, the presence of IL-33 can further promote the decrease in the proportion of exhausted-like subsets.

In summary, IL-33 synergizes with Eomes deficiency to downregulate the expression of Tpex.

3.5 IL-33 Synergizes with Eomes Deficiency to Upregulate the Expression of $Ly108^{-}IFN\text{-}\gamma^{+}CD8^{+}$ T Cells (TEF)

To accurately evaluate the effect of Eomes deficiency and TWS119 on TEF, the TEF proportion in the WT group, WT+IL-33 group, EKO group, and EKO+IL-33 group was compared.

As shown in Figure 5, at 6 h, the TEF proportions in the WT group and EKO group were 2.02% and 4.08%, respectively, a significant increase of 2.06% ($p<0.0001$); at 24 h, the TEF proportions in the WT group and EKO group were 4.72% and 9.06%, respectively, an increase of 4.34%. This indicates that Eomes deficiency can upregulate the proportion of TEF. At 6 h, the TEF proportions in the WT group and WT+IL-33 group were 2.02% and 2.15%, respectively, an increase of 0.13%; at 24 h, the TEF proportions in the WT_CON. group and WT_CON.+IL-33 group were 4.72% and 6.07%, respectively, an increase of 1.35%. This shows that the effect of single IL-33 on TEF is a slight upward trend. At 6 h, the TEF proportions in the EKO group and EKO+IL-33 group were 4.08% and 5.93%, respectively, an increase of 1.85% ($p<0.05$); at 24 h, the TEF proportions in the EKO group and EKO+IL-33 group were 9.06% and 11.1%, respectively, an increase of 2.04%. This indicates that in the case of Eomes deficiency, the presence of IL-33 can further promote the increase in TEF proportion.

In summary, IL-33 synergizes with Eomes deficiency to upregulate the expression of TEFF.

4. Discussion

The precise regulation of $CD8^{+}$ T cell subset differentiation is a core scientific issue in tumor immunotherapy and infectious disease intervention. This study focuses on the synergistic regulatory effect of IL-33 and Eomes deficiency on the differentiation and effector function of $CD8^{+}$ T cell subsets, and reveals the key impacts of the two on the differentiation pathways of stem cell-like memory T cells (TSCM), effector T cells (TEFF), and exhausted precursor cells (TPEX) through in vitro experiments, providing new mechanistic insights for optimizing T cell immunotherapy strategies.

4.1 The Interaction between the Stemness Maintenance Effect of TWS119 on TSCM Cells and Eomes Deficiency

TSCM cells ($Ly108^{+}CD62L^{+}CD8^{+}$), as a "stem cell-like" subset with self-renewal ability and multi-directional differentiation potential, their enrichment is the key to improving the persistence of T cell immunotherapy. This study found that the GSK3β inhibitor TWS119 can significantly upregulate the proportion of TSCM cells, which is consistent with the previous report that TWS119 maintains T cell stemness through the WNT-β-catenin signaling pathway (Front. Immunol. 2023). It is worth noting that

Eomes deficiency (EKO) did not further enhance the promoting effect of TWS119 on TSCM, suggesting that Eomes may not be the main downstream target of TWS119 in regulating TSCM. As a T-box transcription factor, Eomes deficiency leads to downregulated Ly108 expression and increased IFN-γ secretion, indicating that Eomes plays a key regulatory role in the early stage of resting $CD8^+$ T cell differentiation into the effector/exhaustion lineage. Ly108 (SLAMF6), as a common marker of TSCM and TPEX, its downregulated expression may reflect that EKO cells tend to effector differentiation rather than stemness maintenance, which is consistent with the pro-effector function phenotype of IFN-γ.

4.2 The Unidirectional Promotion of IL-33 on Effector T Cell Differentiation and the Inhibitory Effect on Exhausted Precursor Cells

As a member of the IL-1 family, IL-33 has dual functions as a nuclear factor and a cytokine, and it activates the NF-κB and MAPKs signaling pathways through the ST2-IL-1RAcP receptor to drive proinflammatory responses (J. Cent. South Univ. 2021). In this study, IL-33 alone could significantly upregulate the proportion of TEFF cells ($Ly108^-IFN\text{-}\gamma^+CD8^+$), confirming its direct promoting effect on effector T cell differentiation. Mechanistically, IL-33 may accelerate effector function maturation by enhancing IFN-γ gene transcription or stabilizing mRNA expression, synergizing with IL-2/IL-12 stimulation signals. It is worth noting that when IL-33 acts synergistically with Eomes deficiency, the proportion of TEFF cells further increases, while the proportion of TPEX cells ($Ly108^+PD\text{-}1^+CD8^+$) significantly decreases. TPEX, as the precursor cell of exhausted T cells, its reduction may delay the exhaustion process, thereby maintaining the persistence of effector function. This phenomenon suggests that IL-33 not only promotes effector differentiation but also may regulate the fate of $CD8^+$ T cells through a dual-track system by inhibiting the generation of exhausted precursor cells, providing a new strategy for improving the exhaustion of CAR-T cells in chronic infection or tumor microenvironments.

4.3 The Redirecting Effect of Eomes Deficiency on the Differentiation Pathway of $CD8^+$ T Cells

The role of Eomes in $CD8^+$ T cell differentiation is context-dependent: in the virus infection model, Eomes promotes effector T cell differentiation and maintains memory cell survival; while in the tumor microenvironment, T cells with high Eomes expression tend to have an exhausted phenotype. This study found that in EKO cells, Ly108 expression was downregulated, accompanied by increased IFN-γ secretion, indicating that Eomes deficiency may block the differentiation pathway of TSCM/TPEX to the exhaustion lineage, forcing cells to differentiate toward the effector direction. It is worth noting that the promoting effect of IL-33 on TEFF in EKO cells is stronger than that in wild-type (WT) cells, suggesting that Eomes may act as a negative regulator of the IL-33 signaling pathway, or the two share downstream effector molecules (such as T-bet, Blimp-1). In addition, the proportion of $PD\text{-}1^+$TPEX cells in EKO cells significantly decreased under IL-33 stimulation, further confirming that Eomes deficiency and IL-33 synergistically inhibit the generation of exhausted precursor cells, which may be related to Eomes regulating upstream transcription factors of PD-1 (such as TOX, TCF-1).

4.4 The Potential Mechanism and Clinical Transformation Value of Synergistic Action

The core innovation of this study lies in revealing the synergistic effect of IL-33 and Eomes deficiency in the differentiation of $CD8^+$ T cells: the former promotes effector differentiation by activating proinflammatory signals, and the latter inhibits the generation of exhausted precursor cells through transcriptional reprogramming, jointly promoting cells to transform into a high-effector and low-exhaustion phenotype. From the perspective of signaling pathways, the NF-κB/MAPKs pathway of IL-33 may form a cross-talk with the TCF-1/β-catenin pathway regulated by Eomes. For example, Eomes deficiency may enhance the activity of AP-1 transcription factors induced by IL-33, promoting IFN-γ gene expression. In terms of clinical transformation, this study provides new ideas for optimizing CAR-T cell therapy: by knocking out Eomes through gene editing and combining it with IL-33 stimulation, we can enrich CAR-T cell subsets with high effector and low exhaustion in vitro, which is expected to improve their persistence and killing power in solid tumor treatment. In addition, the combined application of small-molecule regulators (such as the GSK3β inhibitor TWS119) targeting the Eomes-IL-33 axis and cytokines may become a key strategy to improve adoptive T cell therapy.

4.5 Research Limitations and Future Directions

Although this study clarified the synergistic effect of IL-33 and Eomes deficiency in an in vitro model, their in vivo effects still need further verification. First, the experiment uses short-term in vitro stimulation (24 hours), and the effects of the two on T cell clonal expansion, metabolic state, and epigenetic modification during long-term culture are still unclear; second, the complex cytokine network (such as TGF-β, IL-10) in the tumor microenvironment may antagonize the effect of IL-33, and the stability of the synergistic effect needs to be evaluated in a tumor-bearing mouse model; finally, whether Eomes deficiency affects the homing ability (such as CD62L expression) and in vivo survival time of T cells still needs to be further studied through adoptive transfer experiments. Future research can focus on the following directions: 1) Construct Eomes conditional knockout mice to analyze the dynamics of $CD8^+$ T cell subsets in vivo; 2) Combine single-cell RNA sequencing and epigenomic analysis to reveal the key transcription modules regulated by the IL-33-Eomes axis; 3) Explore the efficacy and safety of local IL-33 delivery combined with Eomes targeted editing in tumor treatment.

5. Conclusion

This study focuses on the effect of IL-33 and Eomes deficiency on $CD8^+$ T cells, and deeply explores their influence on cell subset differentiation and effector function through in vitro experiments. The study found that TWS119 can significantly upregulate the expression of stem cell-like memory cells (TSCM); Eomes deficiency downregulates the expression of Ly108 and upregulates the expression of IFN-γ; IL-33 alone or in combination with Eomes deficiency can upregulate the expression of effector-like cells (TEFF) and downregulate the expression of exhausted precursor cells (Tpex). These results reveal the synergistic mechanism between IL-33 and Eomes deficiency in the regulation of $CD8^+$ T cell differentiation. This study provides a new theoretical basis for understanding the differentiation and functional regulation of $CD8^+$ T cells. However, it is currently only at the in vitro experimental stage. Follow-up in vivo experiments will be carried out to further explore the effect of IL-33 and Eomes

deficiency on tumor growth in the mouse tumor microenvironment, which is expected to provide new potential targets and treatment strategies for the fields of tumor immunotherapy.

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

The undersigned authors of the manuscript entitled "In Vitro Study on the Synergistic Regulation of $CD8^+$T Cell Subset Differentiation and Effector Function by IL-33 and Eomes Deficiency" confirm that we consent to the publication of this work in *Molecular Medicine*. We irrevocably transfer all copyright ownership of the manuscript, including any tables, figures, or supplementary materials, to the publisher upon acceptance. We authorize the publisher to reproduce, distribute, display, and archive the manuscript in all forms, including print, electronic, and digital formats, and to make such versions available to the public in accordance with the journal's policies. The authors retain the right to use the manuscript for personal or institutional purposes, such as storing it in their own or their institution's repository, provided proper attribution to the original publication is included. We declare that the manuscript is original, has not been published elsewhere, and is not under consideration for publication in another journal. All authors have reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Data Availability/Availability of data and materials

The data can be obtained from Professor Zhu.Email zhuyibei@suda.edu.cn

Competing interests

Not applicable

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Authors' contributions

Li Yan and Xu do the experience. Li wrote the main manuscript text. Yu and Bao prepared all the figures.All authors reviewed the manuscript.

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Figures

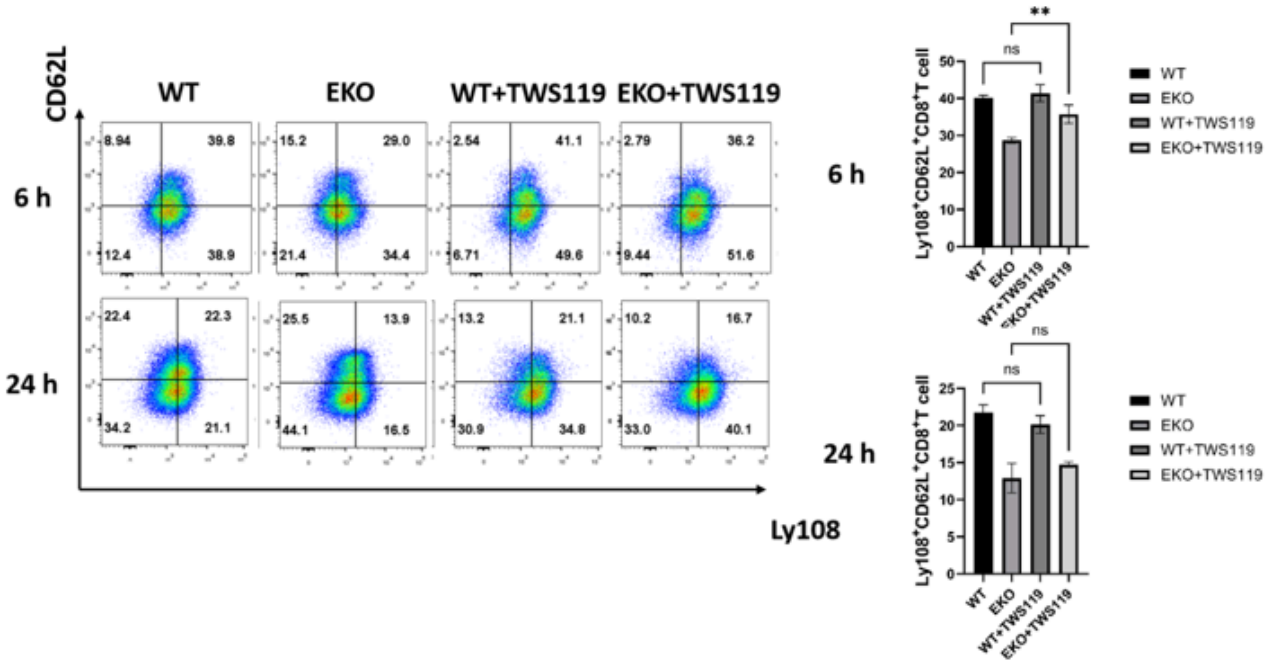

Figure 1

TWS119 upregulates the expression of Ly108⁺CD62L⁺CD8⁺ stem cell-like memory cells (TSCM). This figure shows the effect of TWS119 on the expression of TSCM cells in CD8⁺ T cells of WT and EKO mice. The abscissa is different treatment groups and time points (6 h, 24 h), and the ordinate is the expression level of TSCM cells (Ly108⁺CD62L⁺CD8⁺). The results show that after adding TWS119 in WT and EKO mice, the expression of TSCM cells was upregulated. "ns" in the figure indicates no significant difference, and "**" indicates a significant difference, indicating that TWS119 can effectively promote the expression of TSCM cells, and this effect exists in mice of different genotypes.

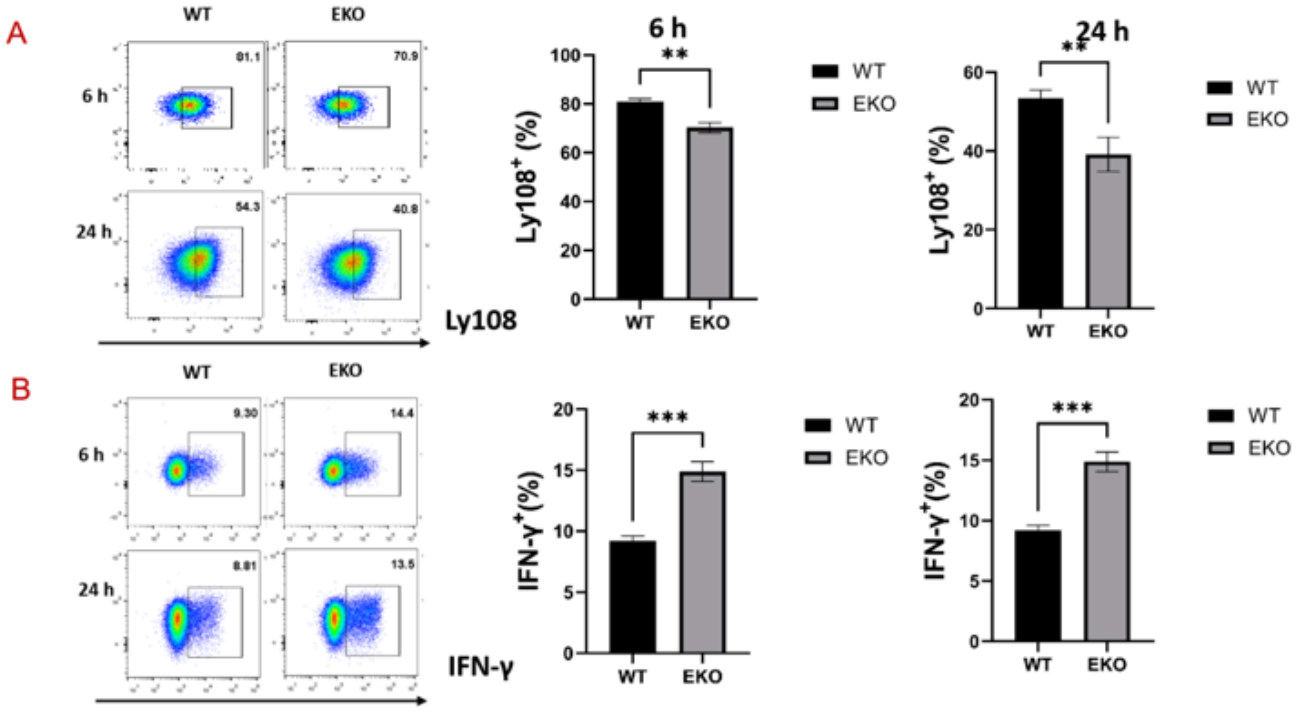

Figure 2

Eomes deficiency downregulates the expression of Ly108 and upregulates the expression of IFN-γ. This figure presents the effect of Eomes deficiency on the expression of Ly108 and IFN-γ in CD8⁺ T cells. The left figure has WT and EKO mouse groups on the abscissa and the expression level of Ly108 on the ordinate; the right figure has the same abscissa and the expression level of IFN-γ on the ordinate. The results show that compared with WT mice, EKO mice have downregulated Ly108 expression and upregulated IFN-γ expression, indicating that Eomes deficiency changes the expression of related molecules in CD8⁺ T cells, affecting cell differentiation and function.

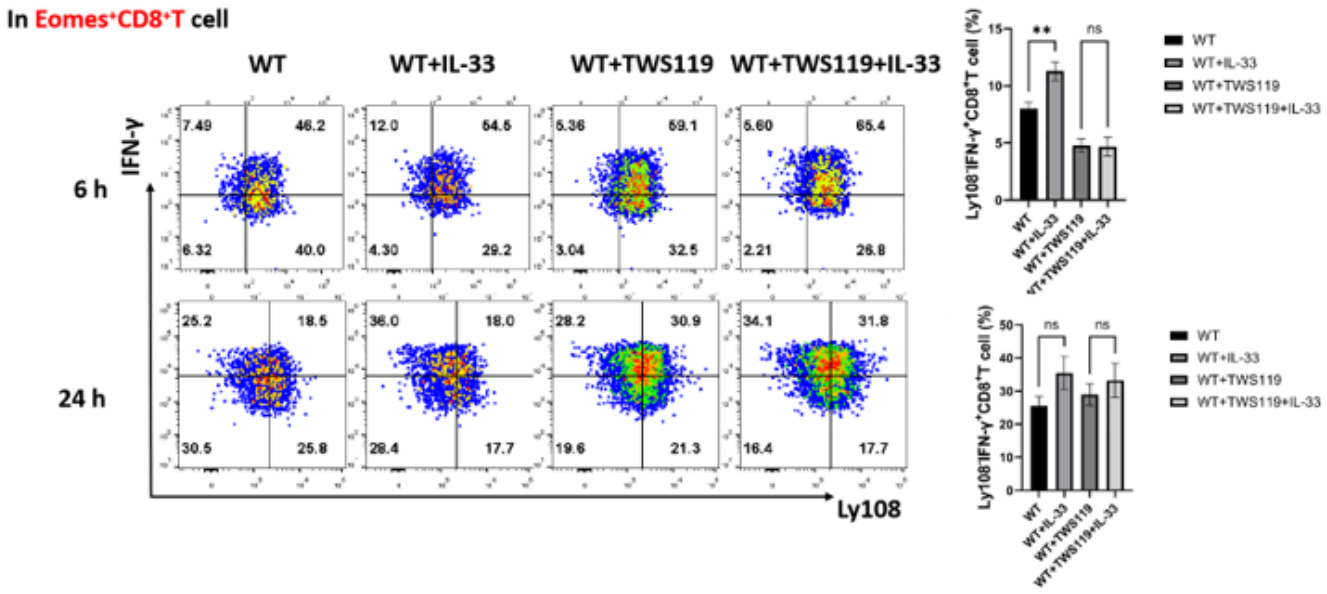

Figure 3

IL-33 upregulates the expression of Ly108⁻IFN-γ⁺CD8⁺ T cells (TEFF). This figure shows the effect of IL-33 on the expression of TEFF cells in WT mouse CD8⁺ T cells. The abscissa is different treatment groups (WT, WT + IL-33, WT + TWS119, WT + TWS119 + IL-33) and time points (6 h, 24 h), and the ordinate is the expression level of TEFF cells (Ly108⁻IFN-γ⁺CD8⁺). The results show that after adding IL-33, the expression of TEFF cells was upregulated. "ns" indicates that the difference between some groups is not significant, and "**" indicates a significant difference, indicating that IL-33 can promote the expression of TEFF cells.

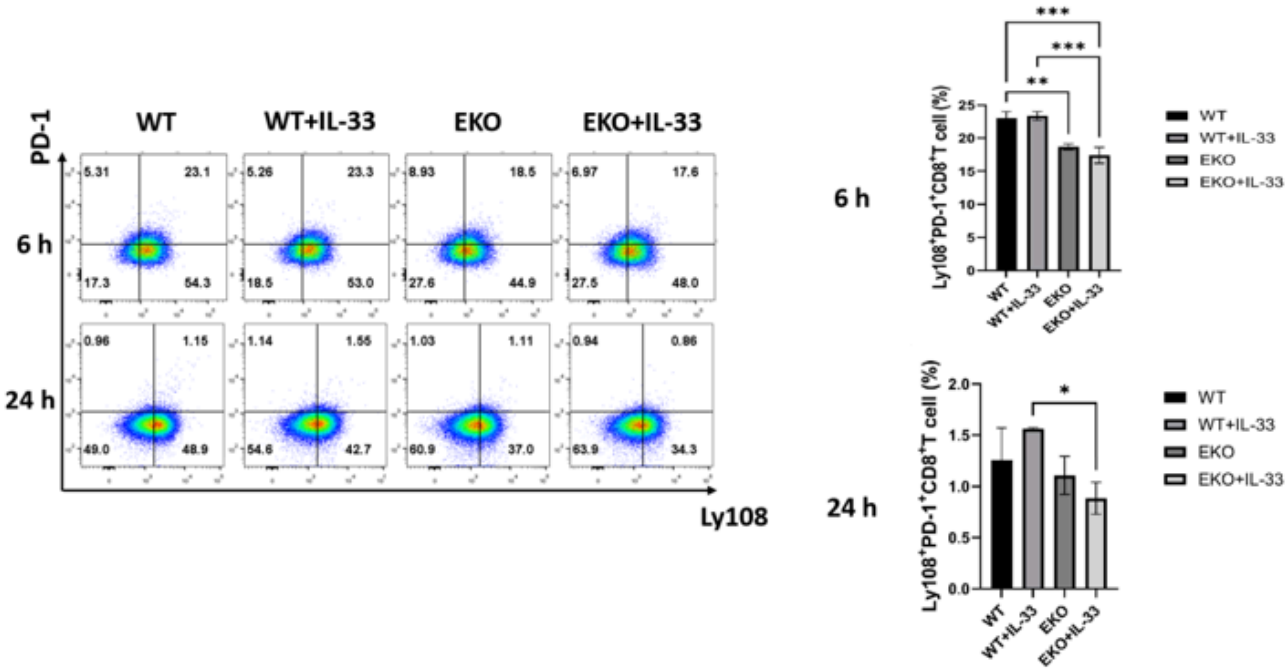

Figure 4

IL-33 synergizes with Eomes deficiency to downregulate the expression of Ly108^{+}PD-1^{+}CD8^{+} exhausted precursor cells (Tpex). This figure presents the effect of the combined action of IL-33 and Eomes deficiency on the expression of Tpex cells in CD8^{+} T cells. The abscissa is different treatment groups (WT, WT + IL-33, EKO, EKO + IL-33) and time points (6 h, 24 h), and the ordinate is the expression level of Tpex cells (Ly108^{+}PD-1^{+}CD8^{+}). The results show that under the combined action of IL-33 and Eomes deficiency, the expression of Tpex cells was downregulated. "***" indicates a very significant difference.

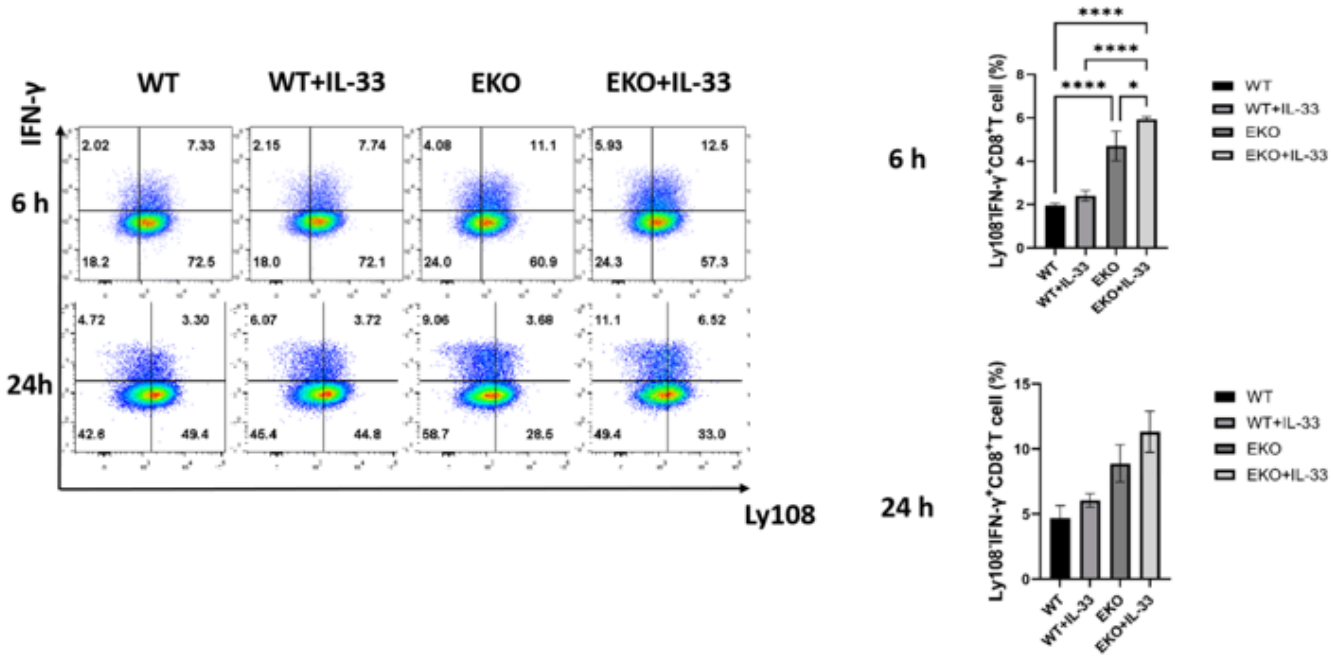

Figure 5

IL-33 synergizes with Eomes deficiency to upregulate the expression of Ly108^{-}IFN-γ^{+}CD8^{+} T cells (TEFF). This figure shows the effect of the combined action of IL-33 and Eomes deficiency on the expression of TEFF cells in CD8^{+} T cells. The abscissa is different treatment groups (WT, WT + IL-33, EKO, EKO + IL-33) and time points (6 h, 24 h), and the ordinate is the expression level of TEFF cells (Ly108^{-}IFN-γ^{+}CD8^{+}). The results show that under the combined action of IL-33 and Eomes deficiency.