LTBR acts as a novel immune checkpoint of tumor-associated macrophages for cancer immunotherapy

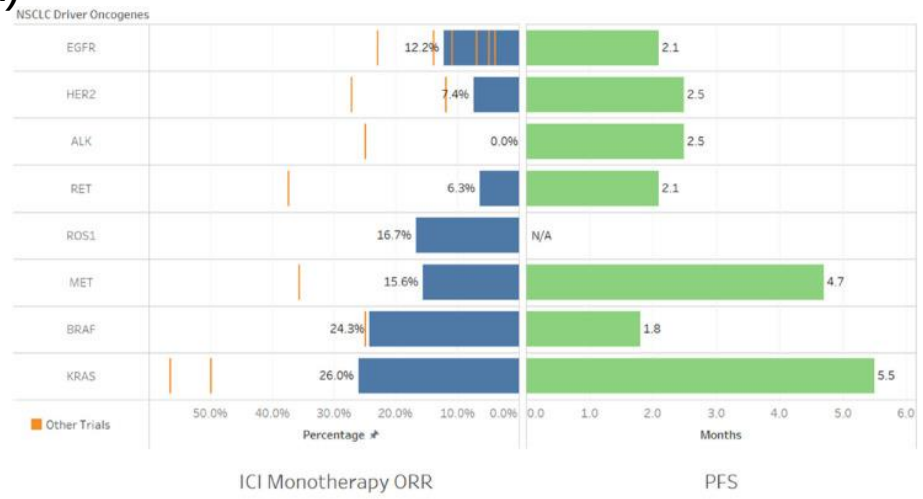
Liang Wang[1,#], Jieyi Fan[2,#], Sifan Wu[1,#], Shilin Cheng[1,#], Junlong Zhao[1], Fan Fan[1], Chunchen Gao[1], Rong Qiao[3], Qiqi Sheng[1], Yiyang Hu[1], Yong Zhang[4], Pengjun Liu[1], Zhe Jiao[1], Tiaoxia Wei[1], Jie Lei[5], Yan Chen[3,*], Hongyan Qin[1,*]

[1] State Key Laboratory of Holistic Integrative Management, Department of Medical Genetics and Developmental Biology, Fourth Military Medical University, Xi'an, 710032, China.
[2] Department of Aerospace Medicine, Fourth Military Medical University, Xi'an,710032, China.
[3] Department of Clinical Oncology, Xijing Hospital, Fourth Military Medical University, Xi'an,710032, China.
[4] Department of Pulmonary Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an,710032, China.
[5] Department of Thoracic Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an, 710032, China.

Liang Wang[1,#], Jieyi Fan[2,#], Sifan Wu[1,#], Shilin Cheng[1,#], Junlong Zhao[1], Fan Fan[1], Chunchen Gao[1], Rong Qiao[3], Qiqi Sheng[1], Yiyang Hu[1], Yong Zhang[4], Pengjun Liu[1], Zhe Jiao[1], Tiaoxia Wei[1], Jie Lei[5], Yan Chen[3,*], Hongyan Qin[1,*]. 2024. LTBR acts as a novel immune checkpoint of tumor-associated macrophages for cancer immunotherapy. ***iMeta*** 3: e233. https://doi.org/10.1002/imt2.233

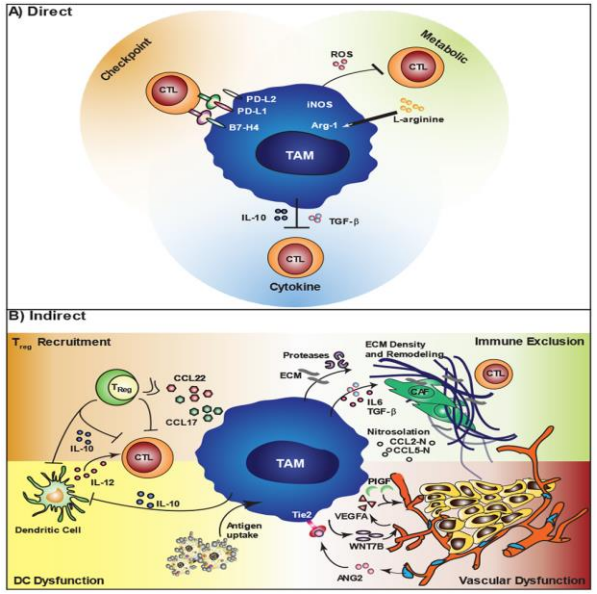
Research background

(A)



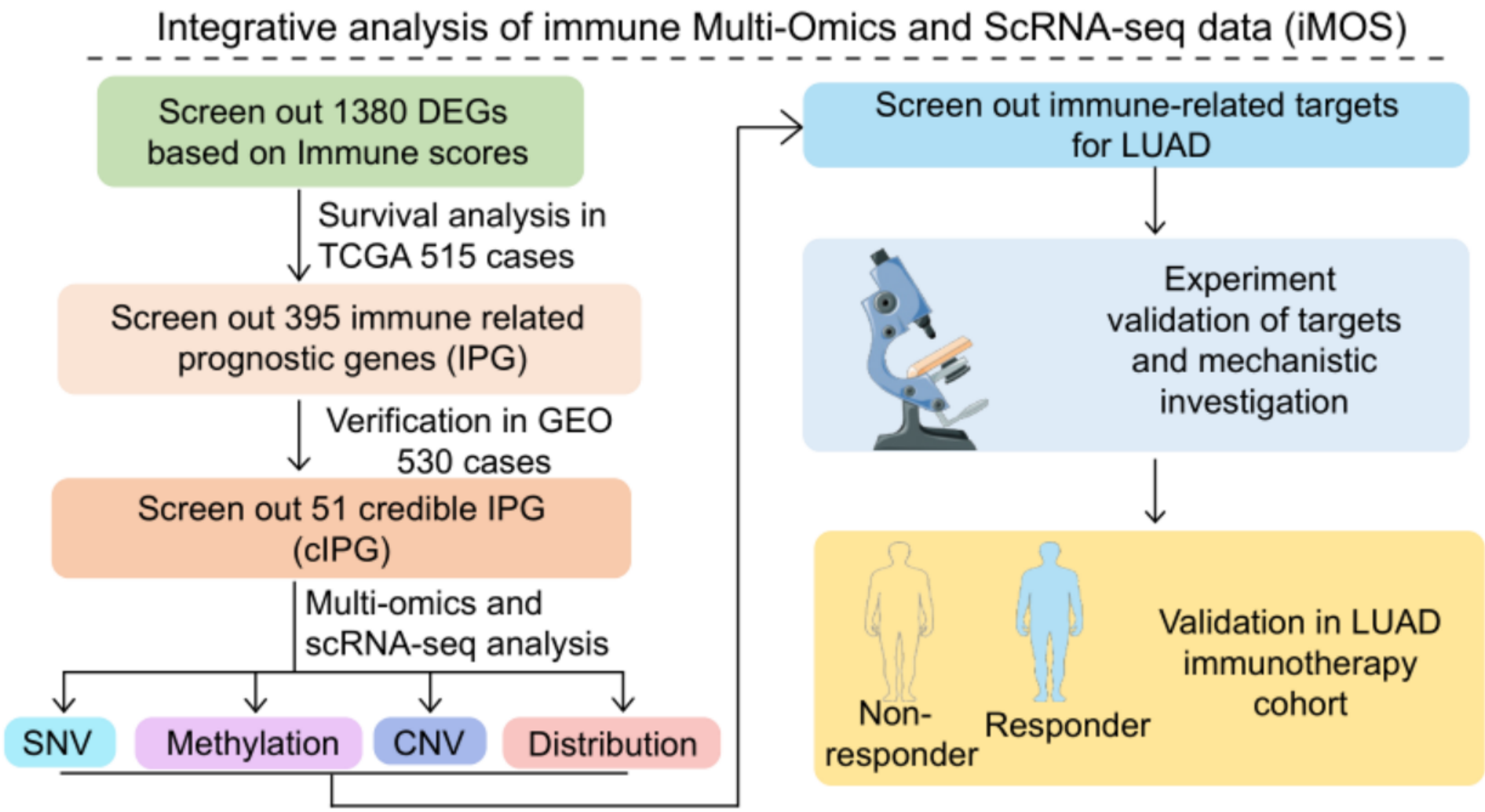
*bars reflect overall ORR (blue) and mPFS (green) demonstrated in retrospective IMMUNOTARGET study
**vertical orange lines depict ORRs shown in other individual ICI monotherapy trials

(B)



- Although immune checkpoint inhibitors (ICI) can significantly improve the event-free survival (EFS) and pathologically complete response (pCR) of lung cancer patients, the overall response rate of ICI in lung cancer patients is only 6.3% to 26%, so it is urgent to reveal the potential mechanism and develop new ICI.
- Tumor immune microenvironment (TIM) plays an important role in tumor initiation, development and immunotherapy resistance.
- Tumor-associated macrophages (TAMs) are the primary cell population of TIM, which not only nourish tumor cells, but also contribute to the tumor immunosuppressive microenvironment (TISM), including depletion of cytotoxic CD8+ T cells and recruitment of immunosuppressive cells such as myelopoietic suppressor cells (MDSC) and regulatory T cells (Tregs).
- Targeting TAMs may be a promising strategy for tumor immunotherapy.

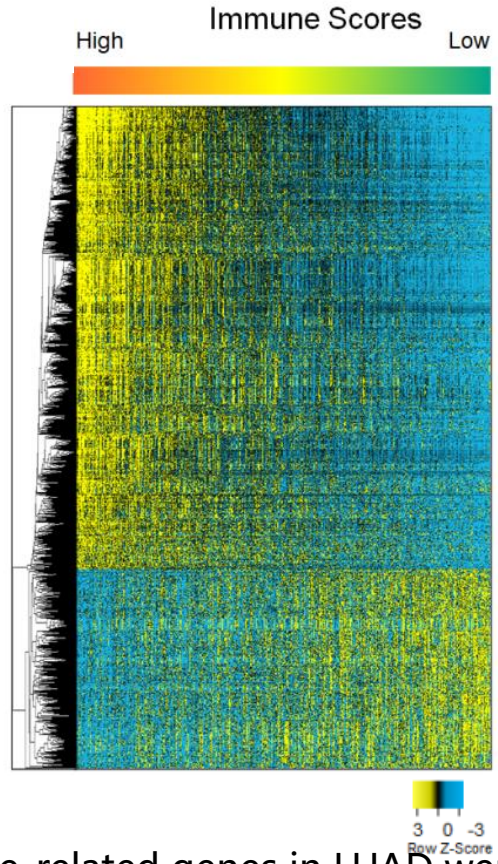
Research results



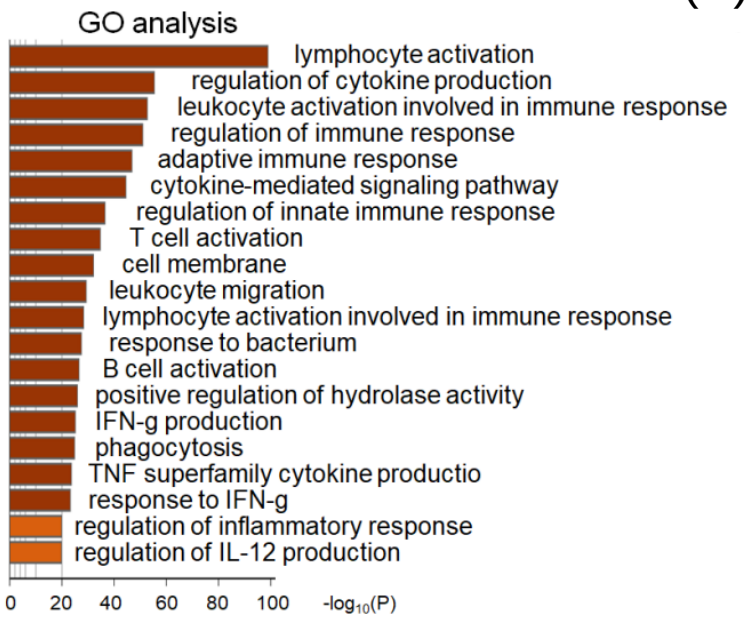
iMOS flow chart of immune checkpoint platform

Research results —Screening out immune-related genes in LUAD by immune scores

(A)



(B)



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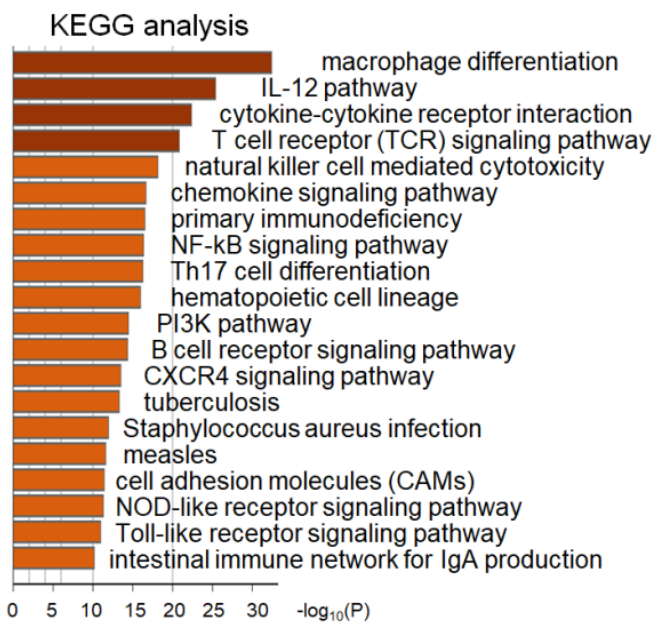
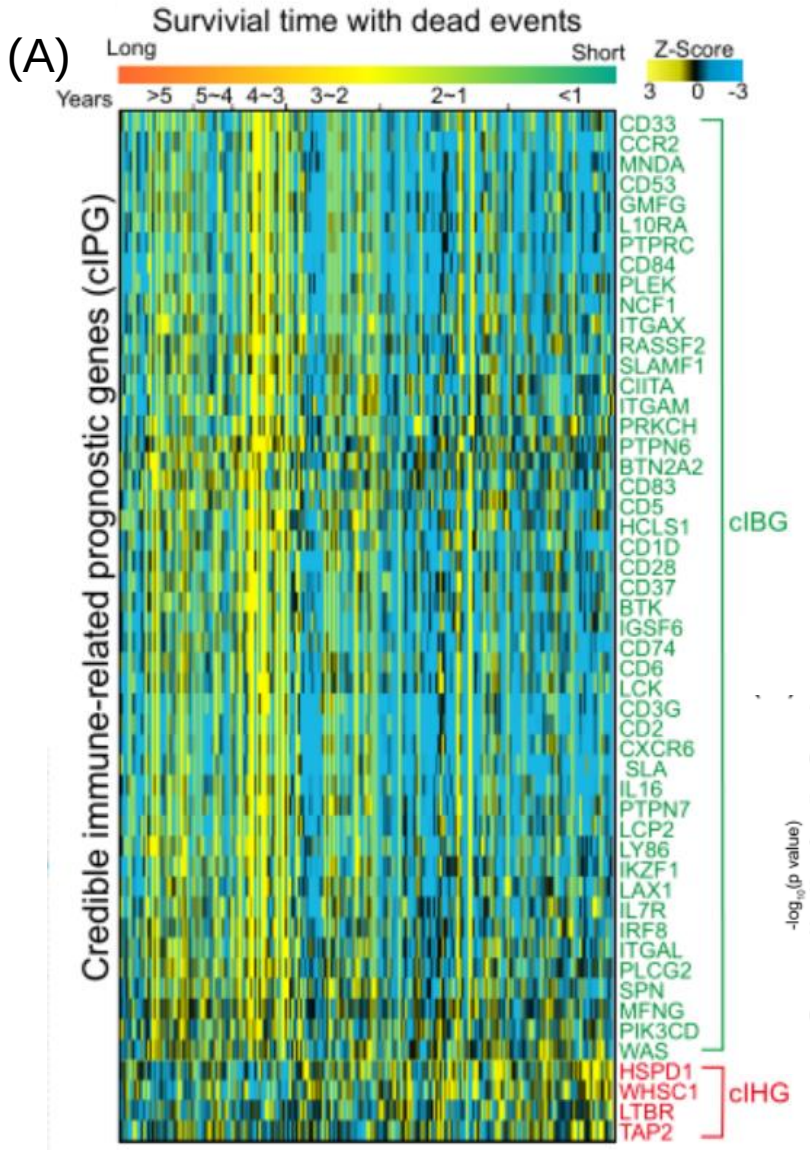


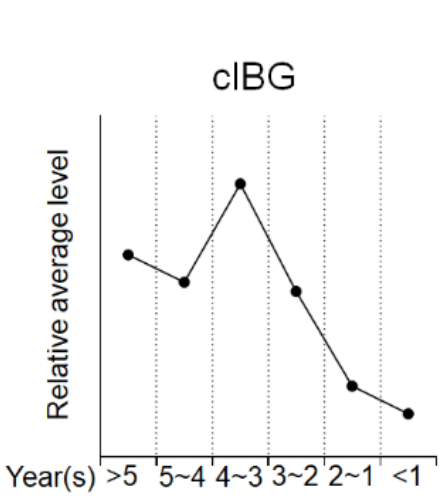
Immune-related genes in LUAD were screened by immune scores to reveal the immune determinants in the LUAD process. 1380 differentially expressed genes were identified, of which 967 genes were up-regulated and 413 genes were down-regulated in the high immunorating group.

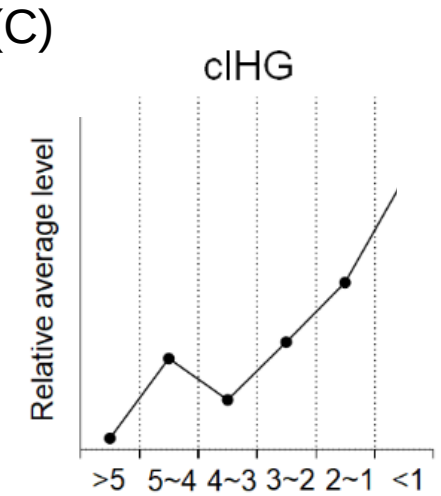
(1) The top five GO enrichment items of these differentially expressed genes were lymphocyte activation, regulation of cytokine production, activation of white blood cells involved in immune response, regulation of immune response, and adaptive immune response.

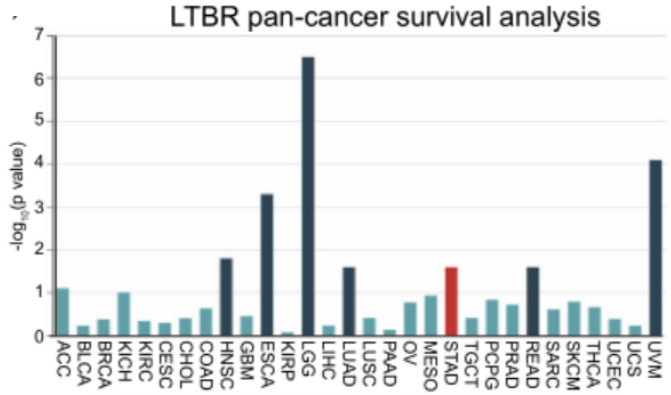
(2) the differentially expressed genes of enrichment of first five KEGG pathways including macrophages, IL-12 pathways, cell factor, cell factor receptor interaction, T cell receptor (TCR) signaling pathway and natural killer cell mediated cytotoxicity.

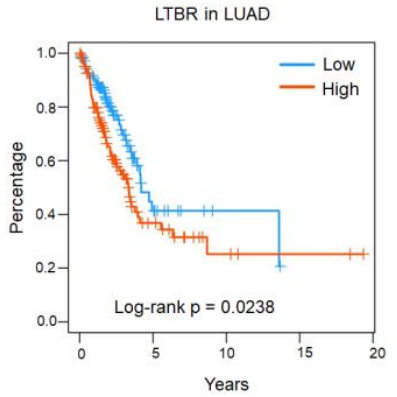
Research results —Screening out credible immune-related prognostic genes in LUAD cohorts

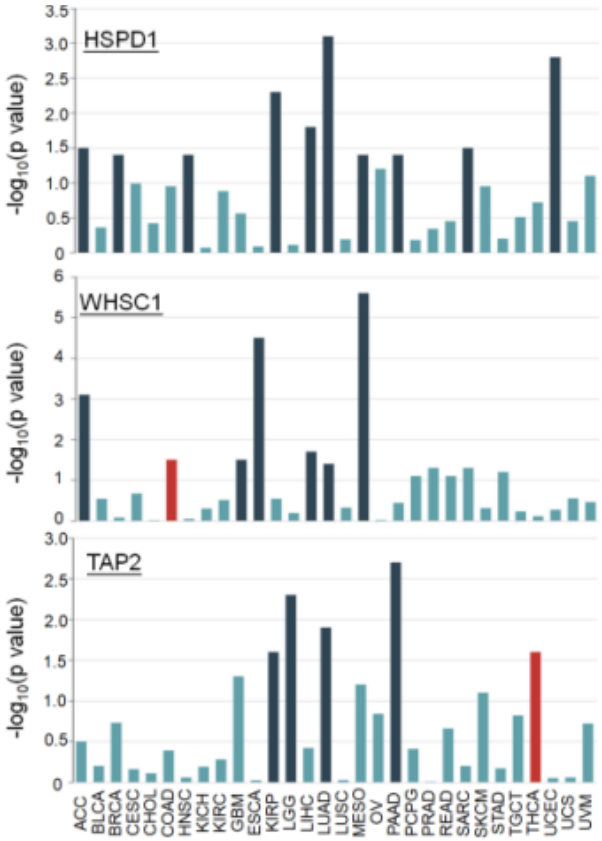








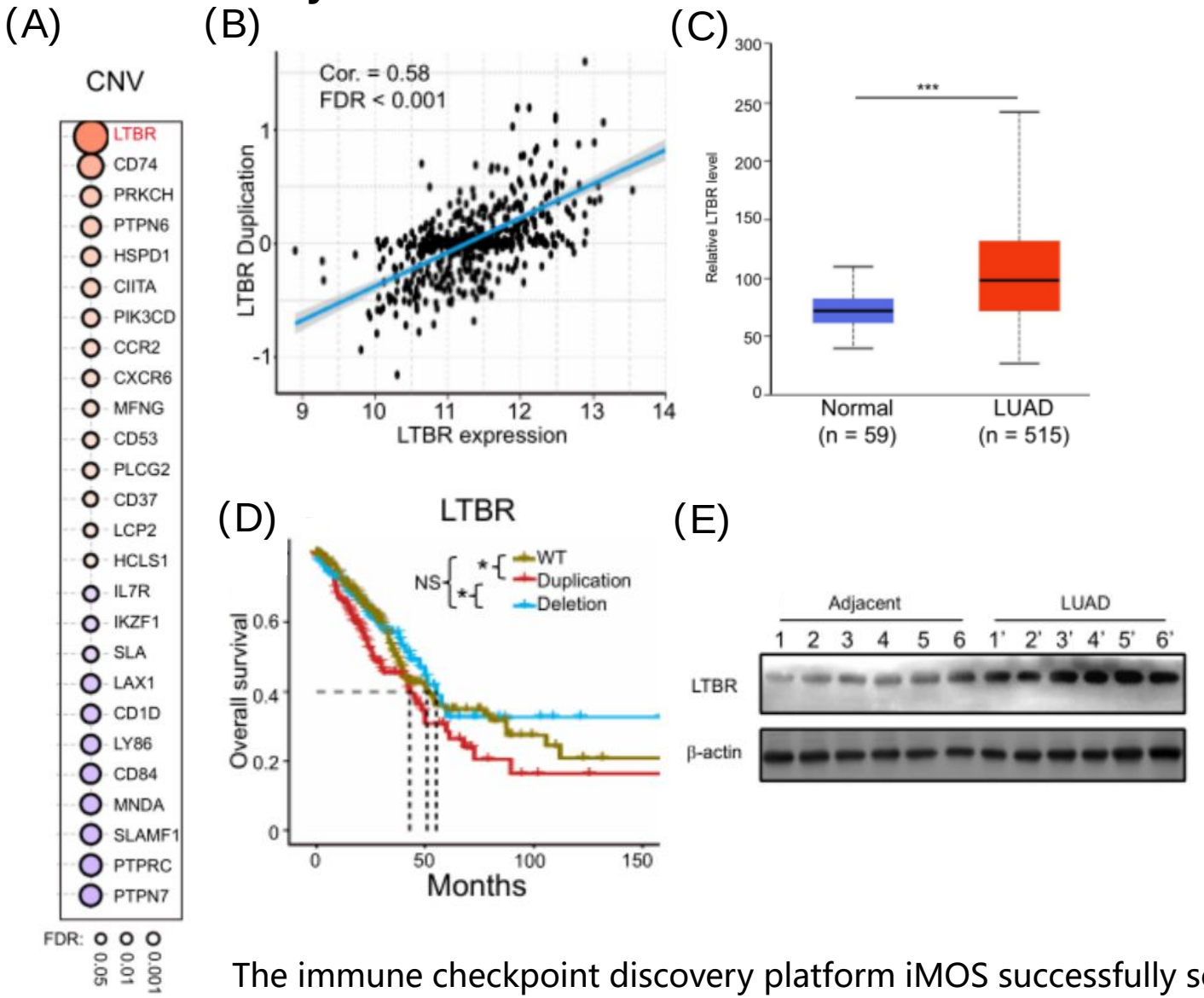




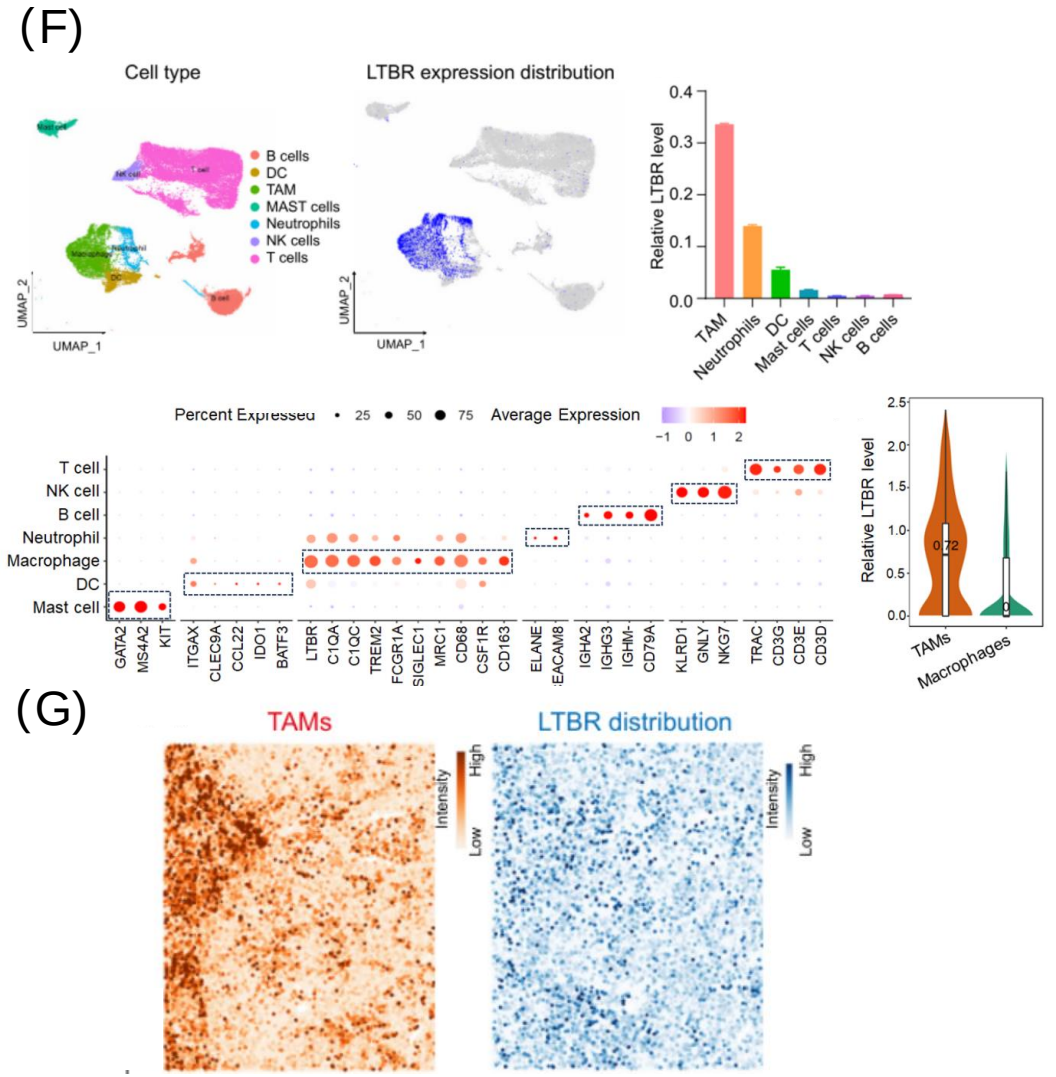
Prognostic significance of IPG in the TCGA cohort in other LUAD cohorts.

Research results —iMOS identifies LTBR as a potential immune checkpoint of TAMs

multi-omics analysis



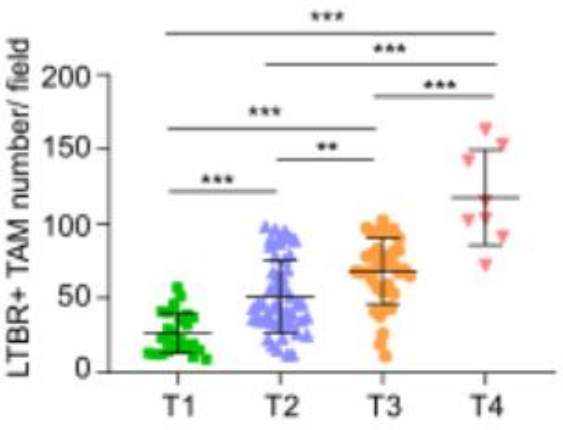
scRNA-seq



The immune checkpoint discovery platform iMOS successfully screened LTBR as a novel immune checkpoint on TAMs.

Research results —LTBR+ TAMs are associated with LUAD stages, immunotherapy failure and clinical prognosis

(A)



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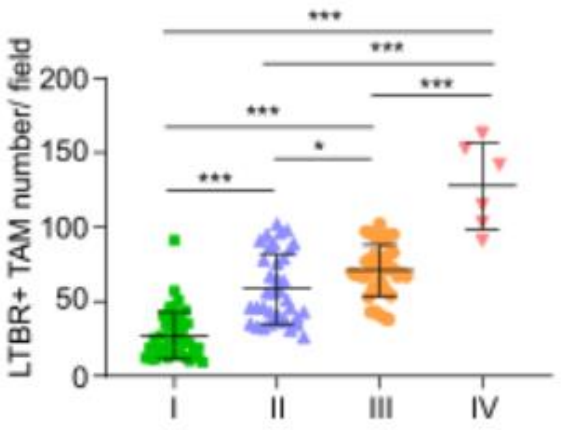
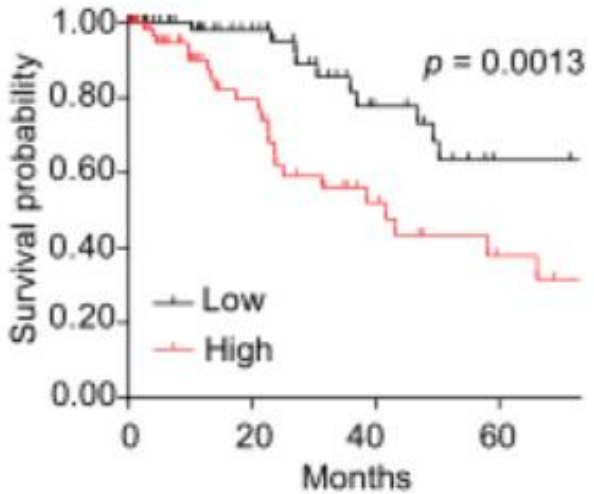


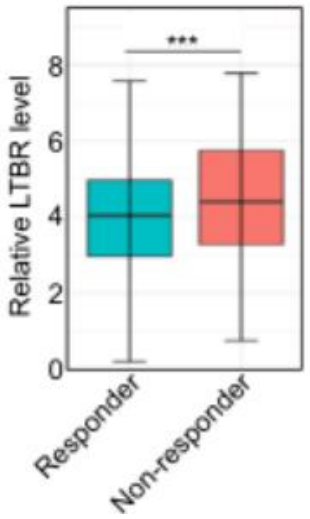
The infiltration of LTBR+ TAMs increased with the malignant degree of LUAD

(C)



Infiltration of LTBR+ TAMs can be used to predict overall survival in patients with LUAD

(D)



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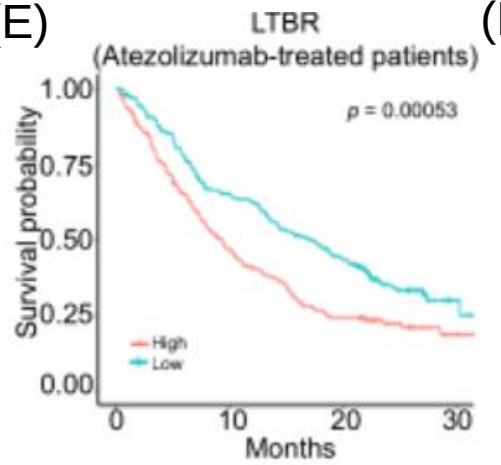

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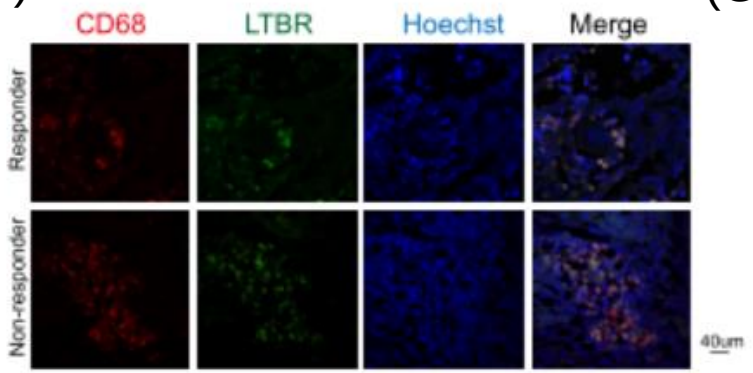
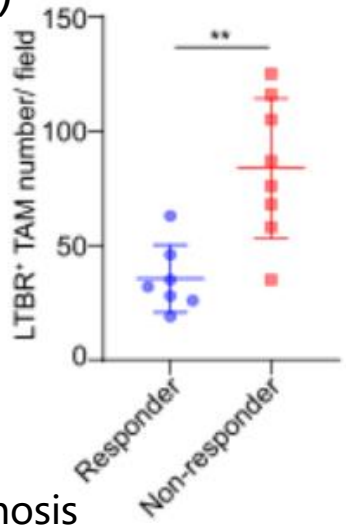
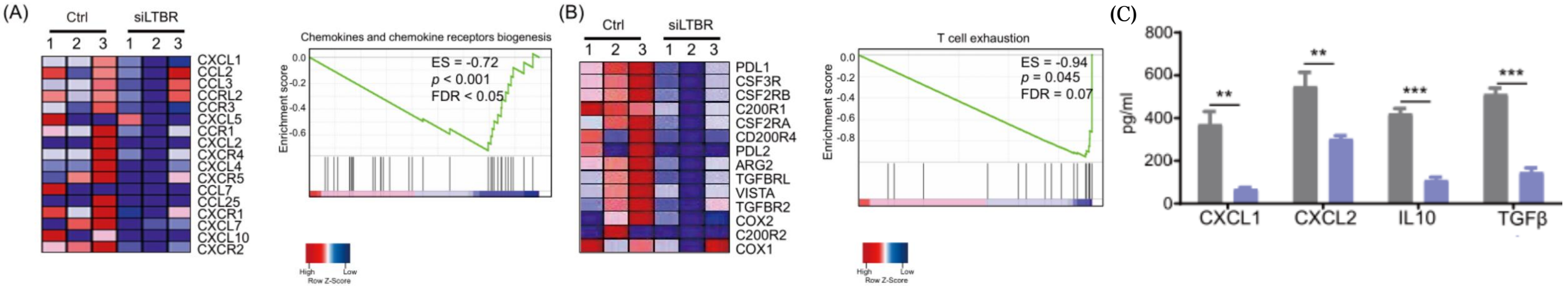


(G)



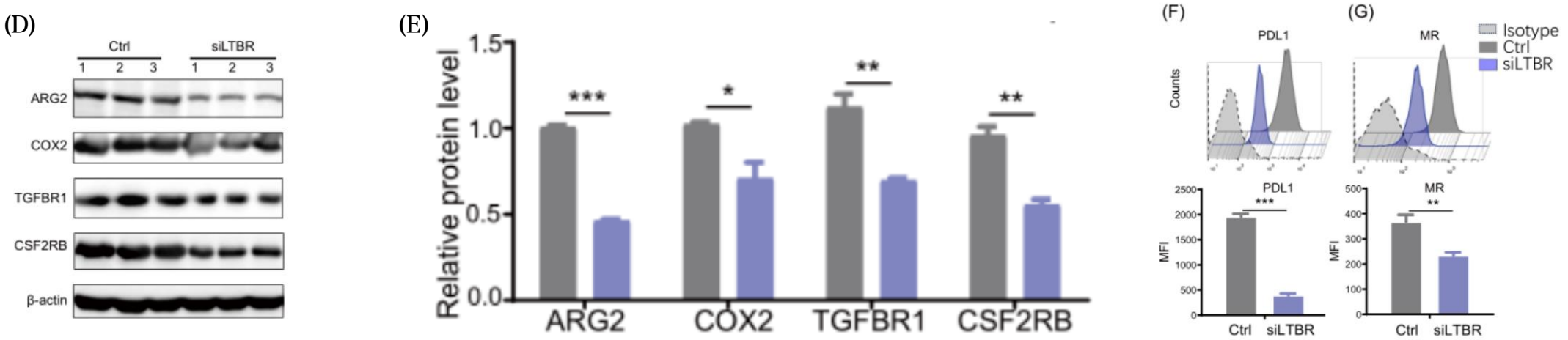
LTBR can be used to predict immunotherapy response and clinical prognosis

Research results —LTBR contributes to maintain TAM-mediated immunosuppression of CD8+ T Cells



GSEA results showed that down-regulation of LTBR inhibited the expression of genes involved in chemokine and chemokine receptor biogenesis and T cell depletion, including CXCL1, CXCL2, PDL1, ARG2, and COX2.

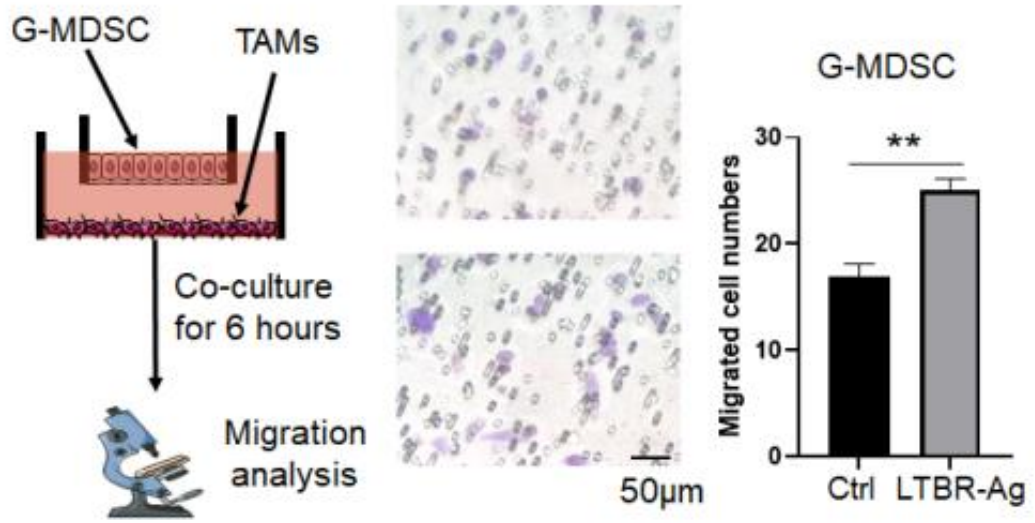
ELISA confirmed that down-regulation of LTBR reduced the secretion of CXCL1, CXCL2, IL10 and TGFb by TAMs.



WB and FACS experiments confirmed that down-regulation of LTBR inhibited the protein levels of PDL1, ARG2, COX2, TGFbR1, CSF2RB and MR In TAMs.

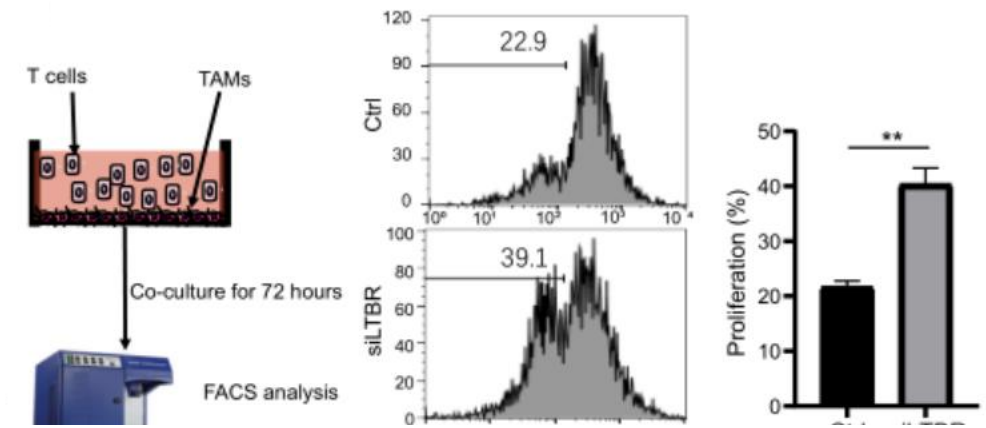
Research results —LTBR contributes to maintain TAM-mediated immunosuppression of CD8+ T Cells

(A)



Activation of LTBR in TAMs promotes recruitment of G-MDSC

(B)



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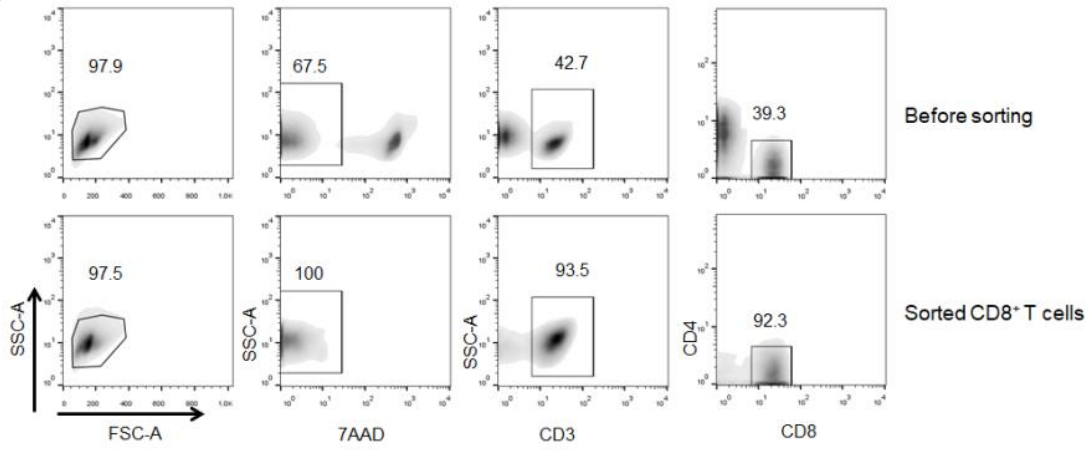
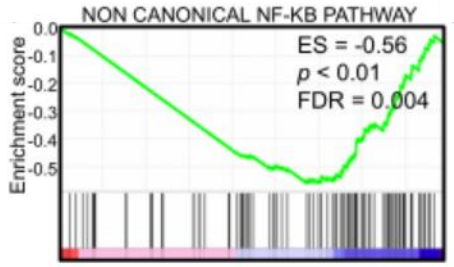


Co-culture experiments of TAMs and CD8+ T cells showed that the destruction of LTBR in TAMs promoted the proliferation of CD8+ T cells.

Research results —LTBR maintained TAM immunosuppressive features and immune escape by non-canonical NF-kB signaling and Wnt/b-catenin signaling

(A)



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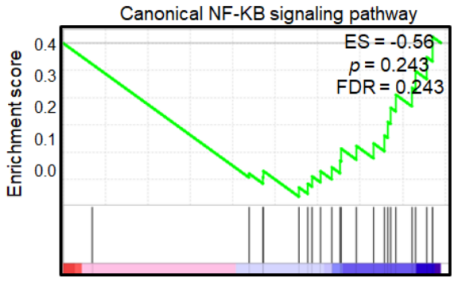
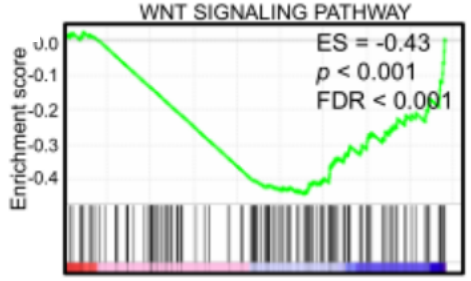


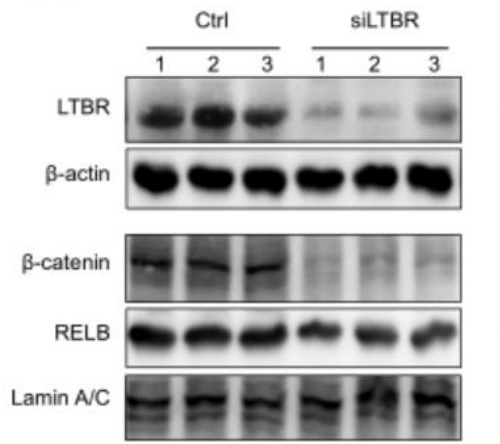
LTBR knockdown in TAMs affects atypical NF-κB and does not affect typical NF-κB signaling

(C)



The knockdown of LTBR in TAMs disrupts the Wnt/ β-catenin signaling pathway

(D)



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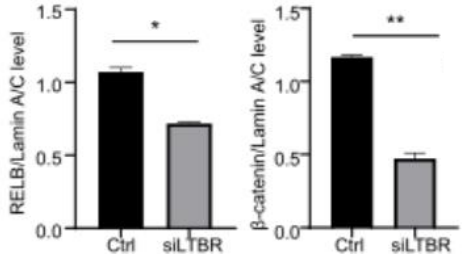
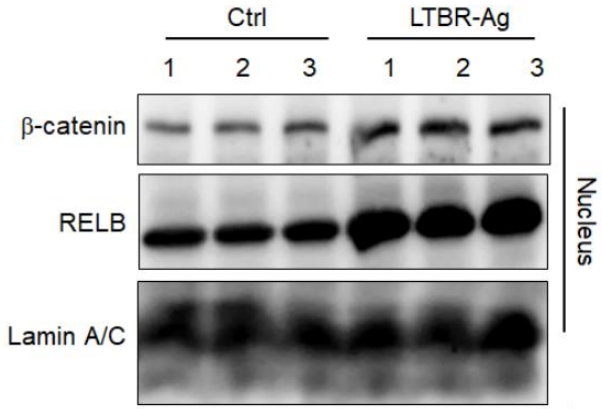


The knockout of LTBR reduces translocation of RELB and β-catenin into the nucleus

(F)



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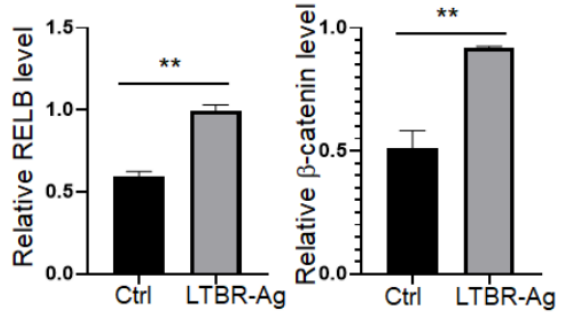
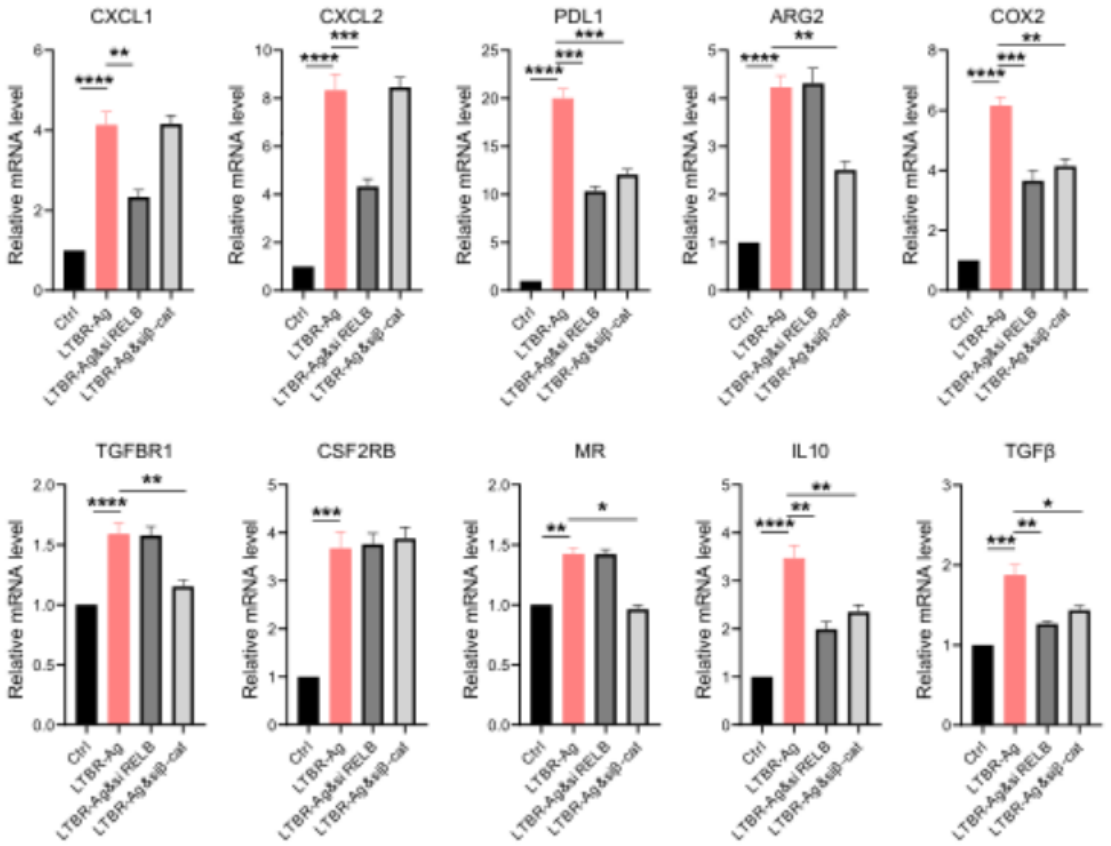


Activation of LTBR promotes translocation of RELB and β-catenin into the nucleus

Research results —LTBR maintained TAM immunosuppressive features and immune escape by non-canonical NF-kB signaling and Wnt/b-catenin signaling

(A)



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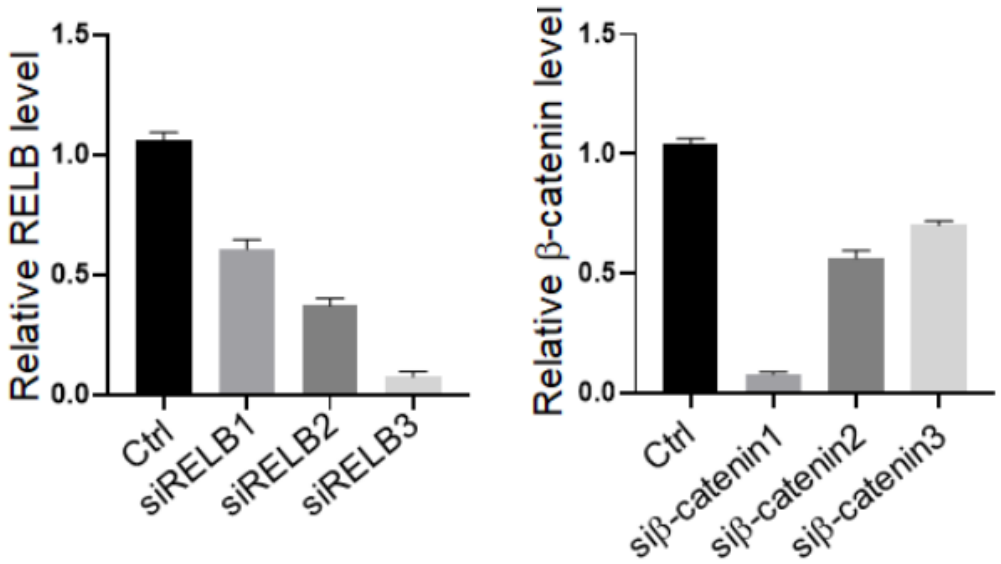
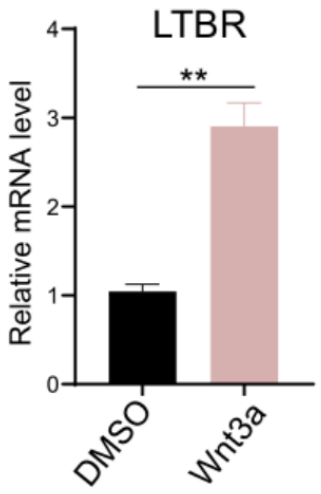


RELB knockout can inhibit the up-regulation of CXCL1, CXCL2, PDL1, COX2, interleukin10 and TGFβ after LTBR activation. Down-regulation of β-catenin attenuates the up-regulation of PDL1, ARG2, COX2, TGFβR1, IL10, Mr And TGFβ after activation of LTBR.

Research results —LTBR maintained TAM immunosuppressive features and immune escape by non-canonical NF-kB signaling and Wnt/b-catenin signaling

(A)



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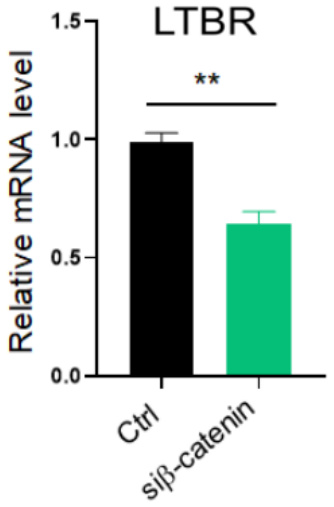

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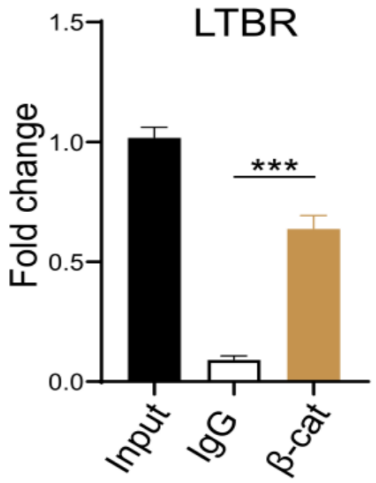

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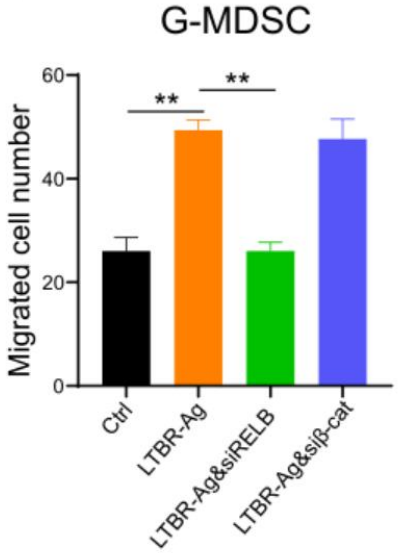

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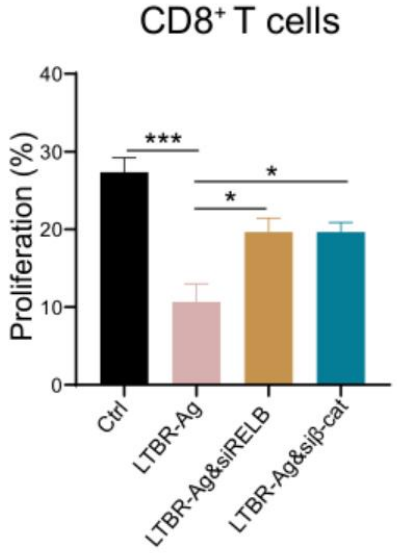
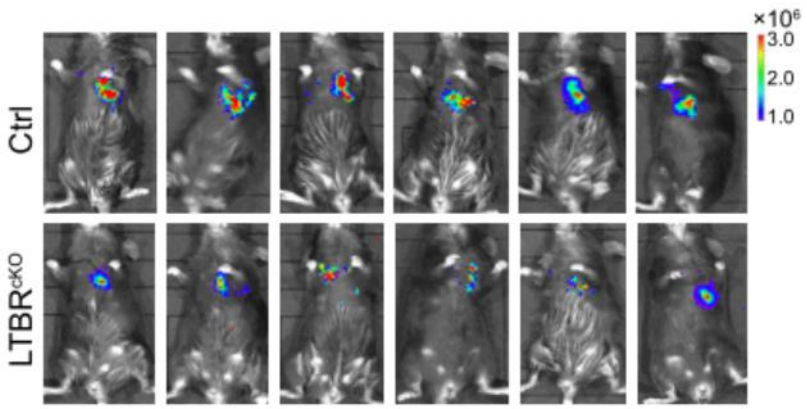


There is a positive feedback regulatory pathway between Wnt/β-catenin signaling pathway and LTBR expression in TAMs, and ChIP experiments have confirmed that β-catenin can bind the promoter region of LTBR.

The deletion of RELB in TAMs and activation of LTBR weakened the recruitment of G-MDSC; Moreover, the co-culture experiments of TAMs and CD8+T cells showed that knockout of RELB or β-catenin could save the proliferation of CD8+T cells after activation of LTBR.

Research results —Knockout of LTBR in TAMs impedes tumor growth via disrupting TAM immunosuppressive activities and M2 phenotype

(A)



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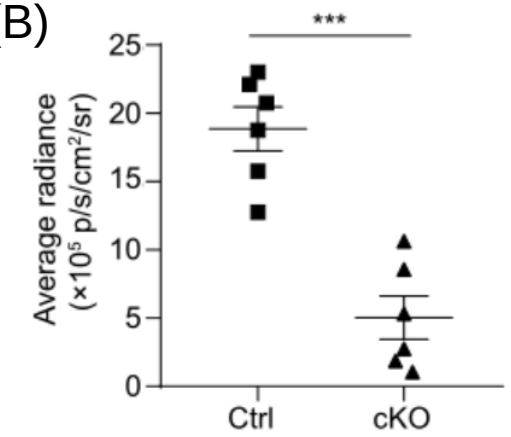

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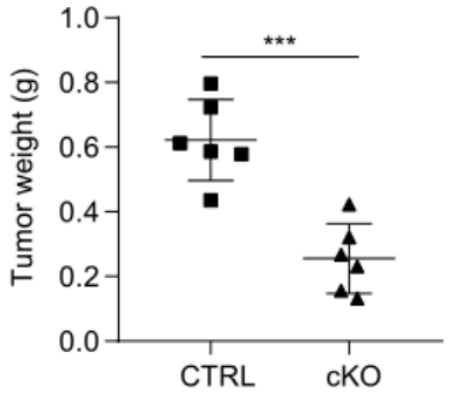
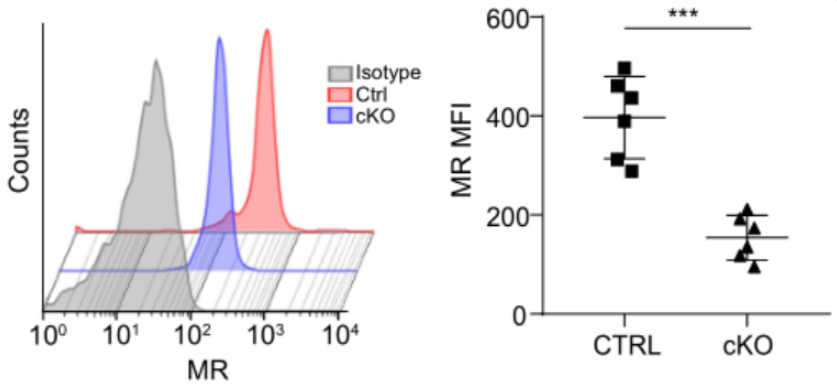


Three weeks after injection of LLC cells, tumor growth in LTBRcKO mice was inhibited compared to Ctrl mice

The tumor weight of LTBRcKO mice was significantly lower than that of Ctrl mice

(D)



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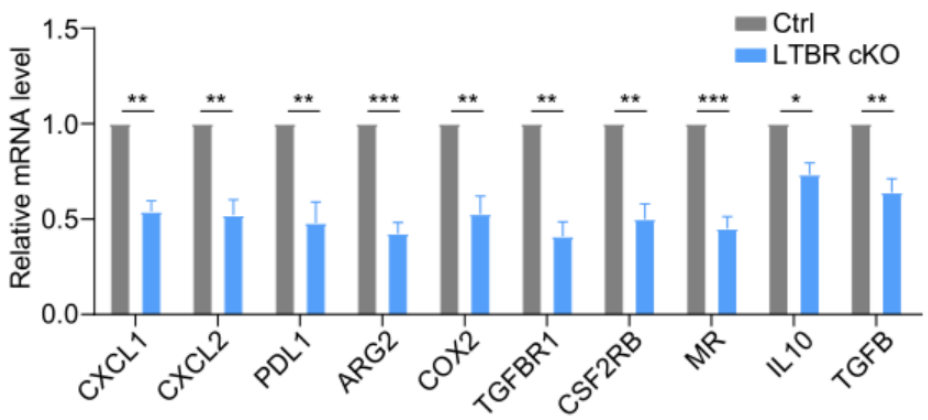
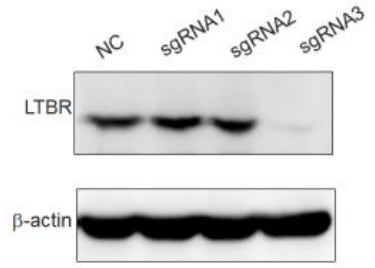


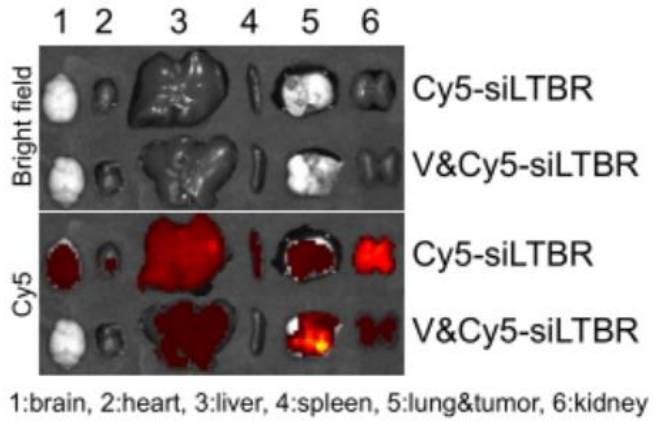
(F)



The deletion of LTBR inhibits the immunosuppressive properties of TAMs and the M2 phenotype

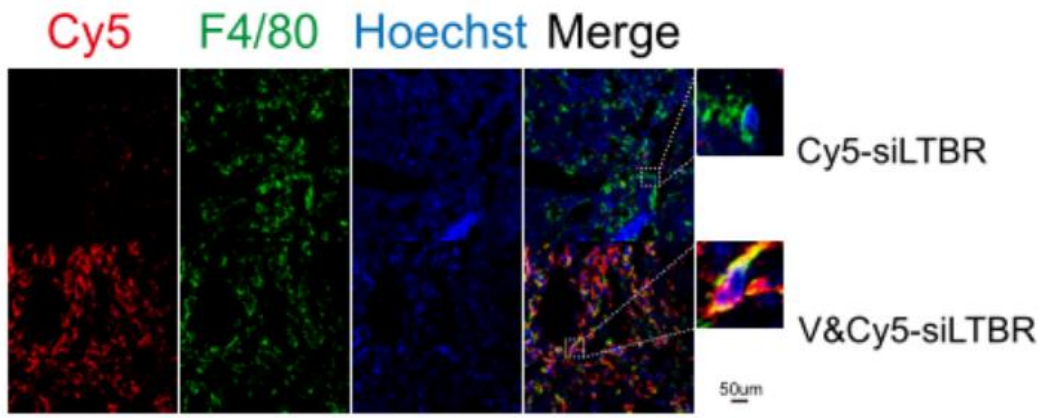
Research results —TAMs-targeted delivery of LTBR siRNA disrupts TAM immunosuppressive ability and improves immunotherapy response

(A)



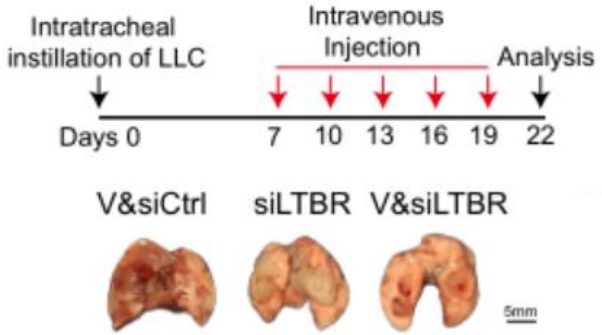
In vivo imaging showed that V&Cy5-siLTBR was mainly enriched in lung cancer tissues

(B)

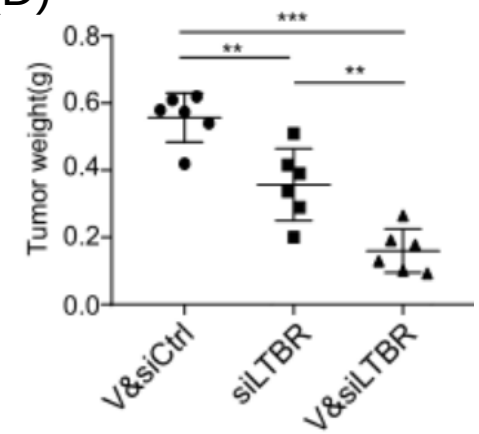


Immunofluorescence assay showed that the system could specifically deliver siLTBR to TAMs

(C)

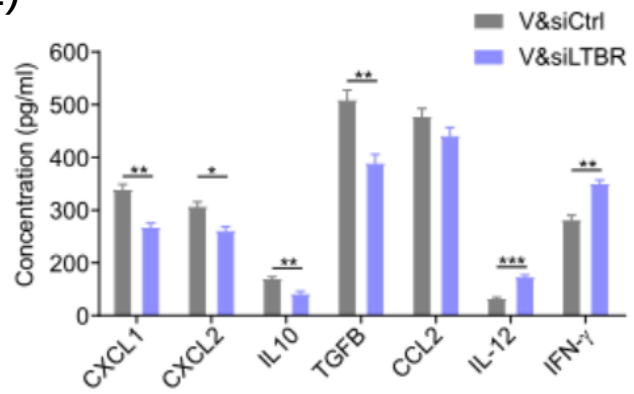


(D)

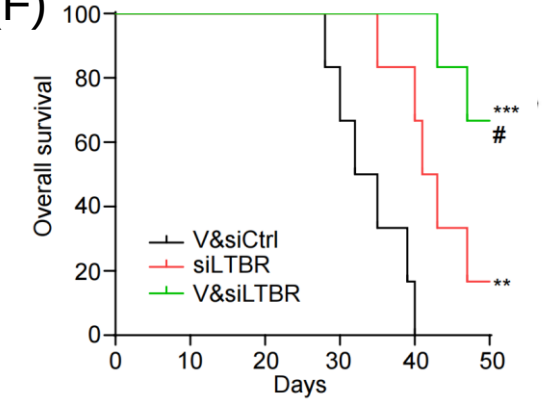


After five treatments, the tumor weight of mice receiving V&siLTBR was significantly lower than that of mice receiving siLTBR or V&siCtrl alone.

(E)



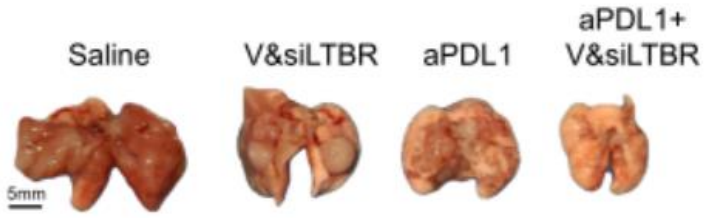
(F)



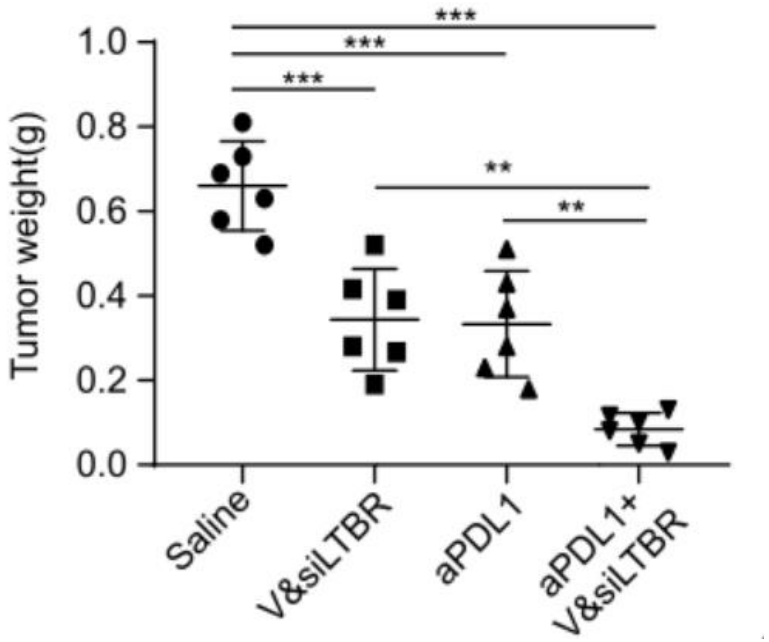
Compared with V&siCtrl, mice treated with V&siLTBR lived longer than mice treated with only siLTBR or V&siCtrl.

Research results —TAMs-targeted delivery of LTBR siRNA disrupts TAM immunosuppressive ability and improves immunotherapy response

(A)

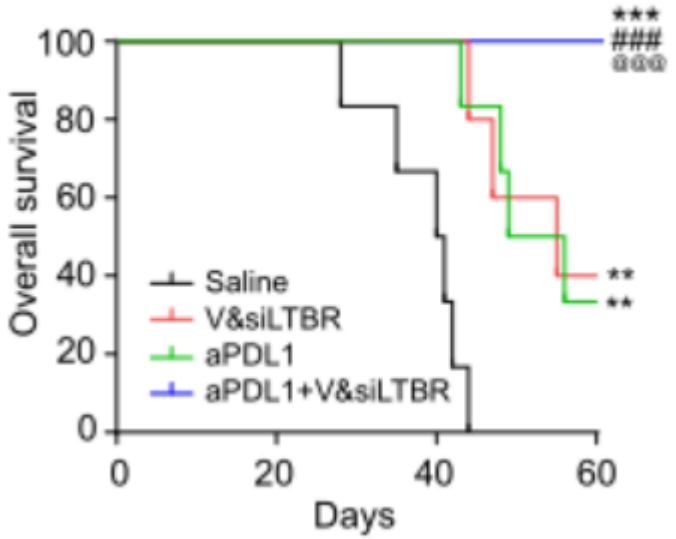


(B)



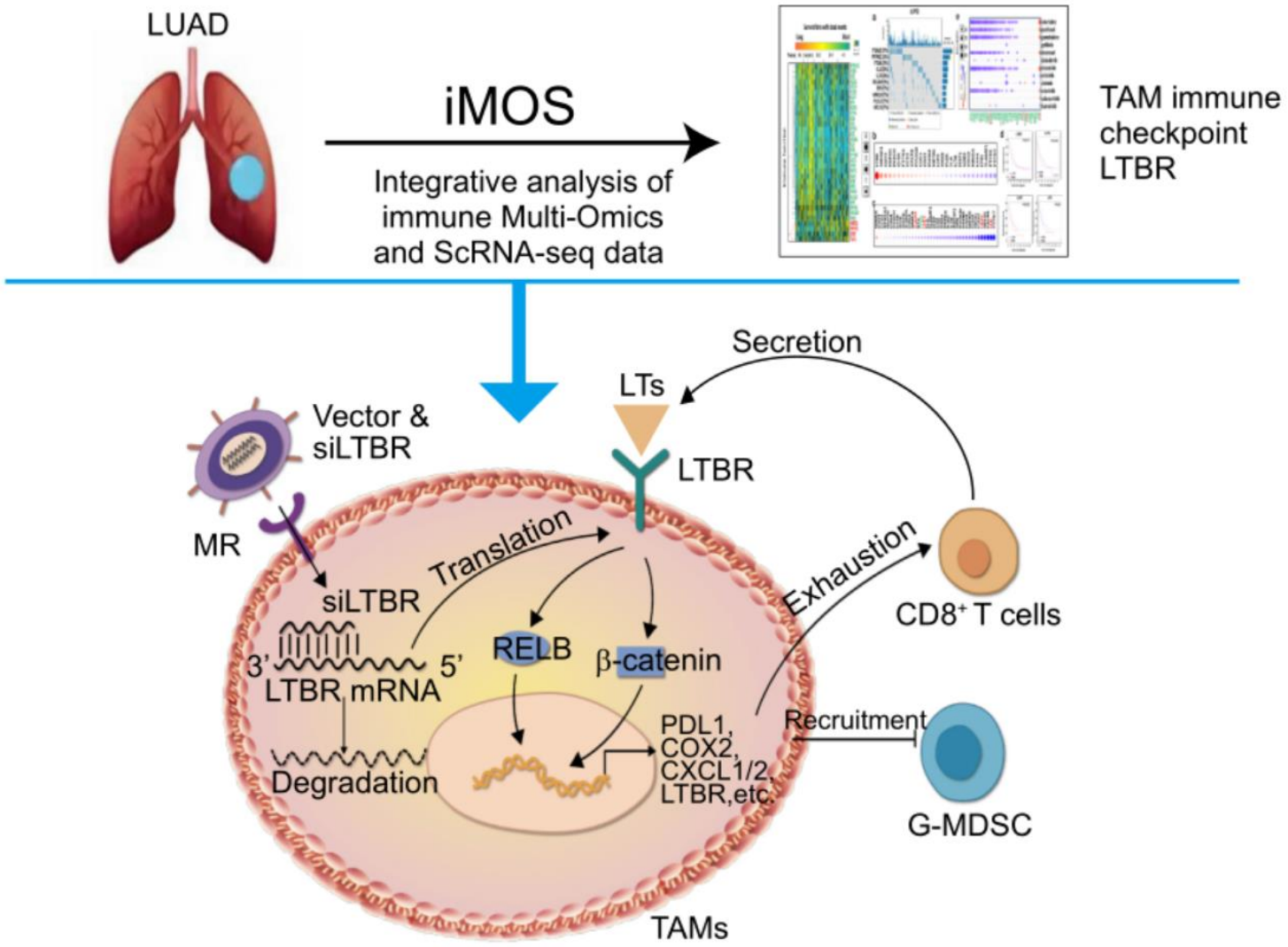
Compared with V&siCtrl, mice treated with V&siLTBR lived longer than mice treated with only siLTBR or V&siCtrl.

(C)



Mice treated with V&siLTBR and aPDL1 lived significantly longer than those treated with V&siLTBR or aPDL1 alone.

Summary of research results



Summary

1. iMOS pipeline is developed and finds LTBR expression relatively specific in TAMs.

2. LTBR+ TAMs correlate with LUAD stages, immunotherapy resistance and prognosis.

3. LTBR maintains TAMs immunosuppressive activity and M2 phenotype by non-canonical NF-κB signaling and Wnt/β-catenin signaling.

4. Disruption of LTBR in TAMs enhances the therapeutic effect of cancer immunotherapy.

Liang Wang[1,#], Jieyi Fan[2,#], Sifan Wu[1,#], Shilin Cheng[1,#], Junlong Zhao[1], Fan Fan[1], Chunchen Gao[1], Rong Qiao[3], Qiqi Sheng[1], Yiyang Hu[1], Yong Zhang[4], Pengjun Liu[1], Zhe Jiao[1], Tiaoxia Wei[1], Jie Lei[5], Yan Chen[3,*], Hongyan Qin[1,*]. 2024. LTBR acts as a novel immune checkpoint of tumor-associated macrophages for cancer immunotherapy. ***iMeta*** 3: e233. https://doi.org/10.1002/imt2.233

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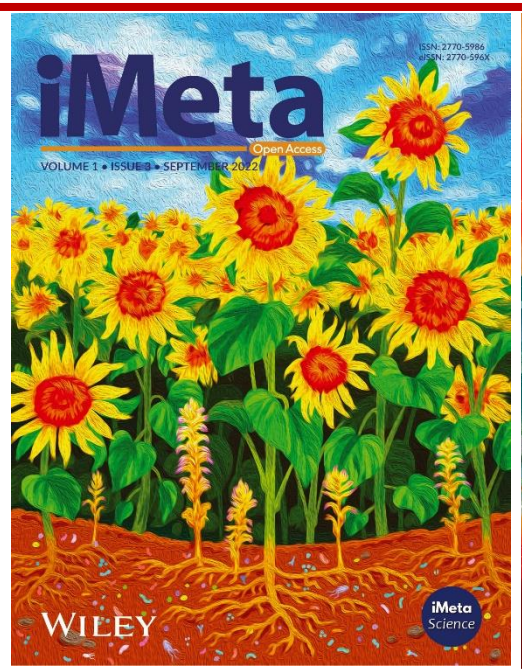

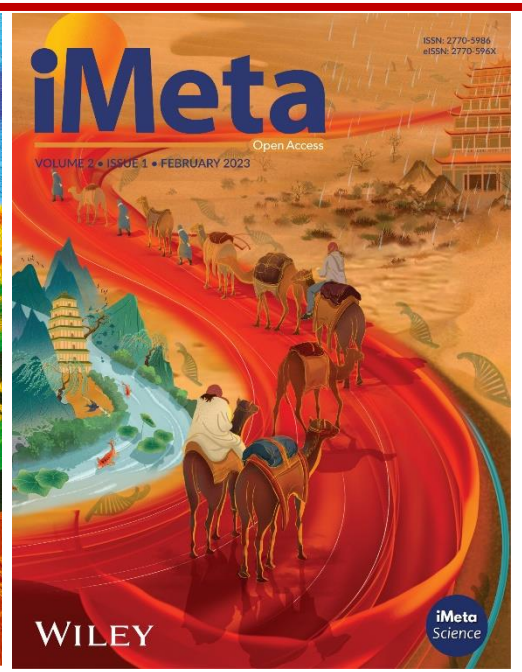

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"***iMetaOmics***" is a sister journal of "***iMeta***" launched in 2024, with a target IF>10, and its scope is similar to *Microbiome, ISME J, Nucleic Acids Research, Briefings in Bioinformatics, Bioinformatics,* etc. All contributes are welcome!

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