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# Blockade of host β2-adrenergic receptor enhances graft-versus-tumor effect through modulating antigen presenting cells

**Hemn Mohammadpour**[*], **Rachel O'Neil**[*,#], **Jingxin Qiu**[‡], **Philip L. McCarthy**[§], **Elizabeth A. Repasky**[*], and **Xuefang Cao**[*,#]

[*]Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

[‡]Department of Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

[§]Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

[#]Department of Microbiology and Immunology, and Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland, Baltimore, MD 21201, USA

## Abstract

Allogeneic hematopoietic cell transplantation (allo-HCT) is a potential curative therapy for hematologic malignancies. Host antigen presenting cells (APCs) are pivotal to the desired graft-versus-tumor (GVT) effect. Recent studies have shown that β2-adrenergic receptor (β2AR) signaling can have an important impact on immune cell functions including dendritic cells (DCs). Here, we demonstrate that pretreatment of host mice with a β2AR blocker significantly increases the GVT effect of donor $CD8^+$ T cells by decreasing tumor burden without increasing GVHD. β2AR-deficient host mice have a significantly increased effector memory and central memory $CD8^+$ T cells and improved reconstitution of T cells including $CD4^+Foxp3^+$ regulatory T cells. Notably, β2AR deficiency induces increased $CD11c^+$ dendritic cell (DC) development. Also, β2AR-deficient bone marrow-derived DCs (BMDCs) induce higher $CD8^+$ T cell proliferation and improved tumor killing *in vitro*. Metabolic profiling shows that β2AR deficiency renders DCs more immunogenic through upregulation of mTOR activity and reducing STAT3 phosphorylation. All together, these findings demonstrate an important role of host β2AR signaling in suppressing T cell reconstitution and GVT activity.

## Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is an established treatment for blood cancers including leukemia and lymphoma. With this treatment, donor T cells are able to attack host tumor cells, producing the beneficial graft-versus-tumor (GVT) effect (1). However, complications may arise in the form of graft-versus-host disease (GVHD) in which donor T cells target normal host tissues resulting in damage to the liver, skin, gastrointestinal tract and other organs (2). This is due to the fact that the donor and the host

**Corresponding author:** Xuefang Cao, MD, PhD, Department of Immunology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, Tel: (716) 845-3963, Fax: (716) 845-1322, Xuefang.Cao@RoswellPark.org.

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The authors have no special/competing interests to disclose.

do not have identical histocompatibility antigens causing donor T cells to recognize the host as foreign (3). However, donor T cell engraftment and reconstitution are also important for protecting the host from infection (4). Therefore, it remains a challenge in allo-HCT to improve T cell reconstitution and GVT effect while achieving control of GVHD.

Host antigen presenting cells (APCs) especially dendritic cells (DCs) are instrumental in inducing GVT and GVHD (5, 6). Tissue damage after cytotoxic conditioning of the host induces DC activation through conditioning-released damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). The activated DCs present host antigens to donor T cells and activate them causing T cell proliferation, differentiation, migration to GVHD target organs, and release of chemokines and cytokines including IFN-γ and IL-2, leading to target tissue damage. Cytotoxic T lymphocytes (CTLs) can directly cause target cell apoptosis during this process (7, 8). The β2-adrenergic receptor (β2AR) is found on APCs including DCs (9), B cells (10) and macrophages (11), T cells (12), and cancer cells (13) and acts as a means of communication between the nervous system and the immune system (14). β2AR activation on DCs results in enhanced anti-inflammatory cytokine production such as IL-10 and decreased IL-6 pro-inflammatory cytokine production *in vitro* (9). Also β2AR activation on bone marrow-derived DCs (BMDCs) shifts $CD4^+$ T cell differentiation towards a Th2 response (15). Our previous report shows β2AR inhibition by β2AR blockers exacerbates GVHD induced by total T cells derived from donor bone marrow and splenocytes, and increased housing temperature also worsens GVHD through decreasing β2AR signaling, indicating an important anti-inflammatory role of β2AR signaling in allogeneic T cell response (16).

In this study, we investigated the effect of β2AR signaling on DC development and subsequent function in GVT effect. Since $CD4^+$ T cells induce hyperacute lethal GVHD making it difficult for GVT study (17), this study has focused on how β2AR signaling in the host affects $CD8^+$ T cell-mediated GVT effect. We demonstrate that β2AR inhibition changes host DC proliferation, function and metabolism which lead to increased T cell reconstitution and enhanced GVT effect without exacerbating GVHD.

## Materials and methods

### Animals and tumor cells

C57BL/6J (H-$2^b$) and BALB/cJ (H-$2^d$) mice were purchased from the Jackson Laboratory. β2 adrenergic receptor knockout (β2AR KO) mice on the BALB/cJ background were provided by J. David Farrar (University of Texas Southwestern Medical Center). All mice were maintained in SPF housing, and all experiments were performed according to the animal care guidelines at Roswell Park Cancer Institute, using protocols approved by the animal studies committee. Luciferase-expressing A20 cells were developed as previously described (18, 19).

### Reagents and antibodies

Antibodies including anti-mouse TCRβ, CD4, CD8, CD44, CD62L, H-$2K^b$, H-$2K^d$, CD122, CD69, CD137, MHC-II, CD86, CD70, CD24, CD172a, and B220 were purchased from

eBioscience. CD90.2 microbeads and negative mouse $CD8^+$ T cells isolation kits were purchased from Miltenyi Biotec and Stem Cell Company respectively. The mouse $CD11c^+$ cell isolation kits for $CD11c^+$ negative selection were purchased from Stem Cell Company.

### Donor cell preparation

Donor bone marrow (BM) cells were isolated from WT C57BL/6 mice. T cell depletion (TCD) was performed by using anti-CD90.2 microbeads (purity >92%). Donor $CD8^+$ T cells were purified from the spleens of C57BL/6 WT by using mouse $CD8^+$ isolation kits (purity >96%).

### Bone marrow transplantation for GVT and GVHD

For GVT studies, β2AR KO and WT BALB/cJ hosts ($H\text{-}2^d$) were irradiated with 900rad from a Cs-137 source at two split doses with 4 hours distance. One day later, the hosts were injected intravenously with $3\times10^6$ TCD-BM cells only or combined with $0.3\times10^6$ $CD8^+$ T cells isolated from C57BL/6 ($H\text{-}2^b$) WT mice. Host mice were injected intravenously with $0.1\times10^6$ luciferase expressing A20 tumor cells right before BM and T cell injection. Tumor burdens were measured by bioluminescence imaging every week and tumor mortality and overall survival were monitored. For β2AR blocking, we performed daily intraperitoneal injection of ICI 118,551, a selective β2AR blocker, before transplantation for 7 days. For GVHD studies, irradiated β2AR KO and WT BALB/cJ hosts ($H\text{-}2^d$) were injected intravenously with $3\times10^6$ TCD-BM cells only or combined with $2\times10^6$ $CD8^+$ T cells isolated from C57BL/6J ($H\text{-}2^b$) WT mice. Then the host mice were weighed every three days and monitored for clinical GVHD score and survival.

### Clinical GVHD scoring criteria

The clinical GVHD manifestations are weight loss; change in posture, activity, fur texture, hair loss and in some cases diarrhea. Clinical GVHD is evaluated comprehensively with a scoring system as published before (17, 20).

### Bone marrow derived dendritic cells (BMDCs) and mixed lymphocyte reaction (MLR)

BMDCs as stimulators were generated from β2AR KO and WT BALB/cJ mice and cultured in 5% RPMI with 1% GM-CSF (GM-CSF releasing cell line supernatant) for 7 days. At day 6, LPS (100ng/ml) were added to mature DCs. Ef670 stained $CD8^+$ T cells as responders were isolated from the spleens of C57BL/6 WT mice. $25\times10^5$ responders and $5\times10^5$ BMDCs as stimulators (ratio 5:1) were co-cultured in 300ul 10% RPMI/well in 96-well plate for 4 to 5 days. Cells were harvested and washed once with 1ml Dulbecco's Phosphate-Buffered Saline (DPBS) before staining for flow cytometry. In tumor killing assay, the luciferase-expressing A20 cells were added in two time points as indicated in the results section.

### Histopathology scoring

30 days after allo-HCT, WT and β2AR KO host mice were sacrificed and the liver, large intestine, and small intestine were removed, fixed with formalin, sectioned, and stained with

H&E. The intestinal tissue was examined using an established semi-quantitative scoring system (19, 21). Representative pictures were captured at 100×.

### Real-time metabolic characterization

An XFe96 extracellular flux analyzer (Seahorse Bioscience) was used to analyze extracellular acidification rate (ECAR, mpH/min) and mitochondria oxygen consumption rate (OCR, $O_2$ mpH/min) in WT and β2AR KO BMDCs. For ECAR analyses, WT or β2AR KO BMDCs were harvested, washed, and re-suspended in ECAR medium (DMEM base [no bicarbonate] with 2 mM L-glutamine, 143 mM NaCl, and 0.5% phenol red [pH 7.35]). The complete ECAR analysis consisted of four stages: basal (without drugs), glycolysis induction (10 mM glucose), maximal glycolysis induction (2 mM oligomycin), and glycolysis inhibition (100 mM 2-DG). For OCR analyses, WT and β2AR KO BMDCs were washed and re-suspended in OCR medium (DMEM base, 25 mM glucose, 1 mM pyruvate, 2 mM L-glutamine [pH 7.35]). Cells were plated in poly-D-lysine coated 96-well flat-bottom plates and incubated in a non-$CO_2$ incubator for 1 hour at 37°C. A complete OCR study was performed with all different groups simultaneously in four consecutive stages: basal respiration (without drugs), mitochondrial complex V inhibition (2 mM oligomycin), maximal respiration induction [1 mM carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)], and electron transportation chain inhibition (1 mM rotenone and 1 mM antimycin A). Immature BMDCs were activated with LPS for either 3 or 18 hours and metabolic analysis were conducted.

### Statistical analysis

Data are presented as mean ± SEM. Difference between groups were analyzed using the unpaired Student's *t* test and one way ANOVA for two and more than two groups respectively. Animal survival (Kaplan-Meier survival curves) was analyzed by log-rank test. The body weight and clinical score difference were analyzed using two way AVOVA and presented by each time point. P-value <0.05 was considered statistically significant. All statistical analyses were performed by using GraphPad Prism v7.

## Results

### Blockade of β2AR in the host increases GVT effect without exacerbating GVHD

It has been shown that host DCs play a pivotal role in inducing GVT and GVHD (5). β2AR signaling contributes to regulating DC functions and subsequent T cell activation (22). Previous reports showed that β2AR inhibition increases inflammatory phenotype of DCs (23) and β2AR agonists bias DC function towards inducing an IL-17 immune response (24). In order to address the effect of β2AR deficiency in host DCs on GVT activity, we used two different methods: 1) pretreatment the host mice with the β2AR blocker (ICI 118,551) for 7 days before transplantation and 2) using WT and β2AR knockout (β2AR KO) BALB/cJ ($H\text{-}2^d$) mice as hosts. C57BL/6J ($H\text{-}2^b$) mice were used as donors. Host mice were lethally irradiated and inoculated with $0.1\times10^6$ A20 cells before transplanted with $3\times10^6$ TCD-BM and $0.3\times10^6$ $CD8^+$ T cells from B6 donors. Representative images of tumor burden are shown in Figure 1A. Pretreatment was performed for 7 days, daily injection with 5mg/kg ICI-118,551 before transplantation. The group pretreated with the β2AR blocker had

significantly decreased tumor burden than the PBS control group, and showed a strong trend of improved survival (Figure 1B). In parallel, weight loss of these mice was used as readout for GVHD. Both the blocker pretreatment group and the PBS control group were able to recover to their original body weight before some mice succumbed to tumor outgrowth. These observations were confirmed by using β2AR KO hosts, which showed significantly decreased tumor burden in comparison to WT hosts (Figure 1C). Interestingly, β2AR KO hosts also showed significantly improved bodyweight recovery in comparison to WT hosts (Figure 1C). Notably, β2AR KO hosts showed significantly improved survival (100%) compared to WT hosts (60%). As controls, both WT and KO groups receiving BM only showed uncontrolled tumor growth and 100% tumor-induced mortality. To understand the underlying mechanisms, we first examined donor-derived $CD8^+$ T cells at different time points after allo-HCT (Supplemental Figure 1). At all three time points, days 10, 30 and 60, there were significantly higher numbers of $CD8^+$ T cells in β2AR KO hosts compared to WT hosts (Supplemental Figure 1A). We used CD44 and CD62L to assess effector and memory phenotypes. At day 10, nearly 95 percent of $CD8^+$ T cells were effector cells in both WT and β2AR KO hosts (Supplemental Figure 1B). However at day 30, compared to WT hosts, there was a significant increase in $CD44^+CD62L^-$ effector memory cells in β2AR KO hosts (Supplemental Figure 1B). Interestingly, at day 60, the $CD44^+CD62L^+$ central memory cells were significantly increased in β2AR KO hosts compared to WT hosts. Together, these data demonstrate that β2AR deficiency in the host enhances $CD8^+$ T cell expansion and memory formation, resulting in improved GVT effect without aggravating GVHD,

## β2AR suppresses dendritic cell (DC) development and function

Catecholamine including dopamine, norepinephrine and epinephrine has both neural and endogenous sources in bone marrow and affects hematopoiesis (25). Almost all immune cells including macrophages, monocytes, T cells and B cells can synthesize and release catecholamine and change function via autocrine loop (26–28). Host APCs are crucial for inducing both GVHD and GVT (7). Furthermore, it has been demonstrated that different subpopulations of DCs have different roles in induction of GVHD and GVT. Host $CD8^+$ DCs have a protective role in GVHD and are important for the optimal GVT effect (29). Also, some populations of plasmacytoid DCs exacerbate GVHD symptoms (30), yet CCR-$9^+$ plasmacytoid DCs are able to suppress GVHD (31). Since our results indicate a role for β2AR in GVT, we investigated the effect of β2AR deficiency on $CD11c^+$ DC development and function. β2AR KO mice have higher percentages of $CD11c^+$ population but there was no difference in $CD11c^+$ subpopulations including plasmacytoid DCs, $CD11b^+$ DCs and $CD8^+$ conventional DC identified with B220, CD172a and CD24 markers respectively (Figure 2A). To test the effect of β2AR deficiency on DC function, we developed BM-derived DCs (BMDCs). Interestingly, when we started with the same number of BM cells, β2AR KO BM cells yielded a higher number of BMDCs after 6 days of culture compared to WT BM cells. Yet there is no difference in $CD11c^+$ percentage between WT and β2AR KO BMDCs (Figure 2B). When we examined the quality of the BMDCs, the KO BMDCs displayed significantly higher CD86 and MHC-II expression than the WT BMDCs (Figure 2C). Next we assessed cytokine production by BMDCs after LPS maturation. WT BMDCs released more IL-10 compared to KO BMDCs and that ratio of IL-10/IL-12 also

was significantly higher in WT compared to KO BMDCs (Figure 2D). We then conducted a mixed lymphocyte reaction (MLR) using the BMDCs as stimulator cells and ef670-stained $CD8^+$ T cells as responder cells. Compared to WT BMDCs, β2AR KO BMDCs induced a significant increase in T cell proliferation (Figure 2E). Also, we checked the expression of CD122 as an activation marker and there were a significantly higher percentage of $CD122^+$ T cells activated by the β2AR KO DCs on day 4 and day 5 (Figure 2F). There were also significantly higher percentages of effector cells marked by $CD44^+CD62L^-$ T cells activated by β2AR KO DCs on both days 4 and 5 compared to WT BMDCs (Figure 2F). Together, these data indicate that β2AR suppresses DC development and function.

## β2AR deficient BMDCs show decreased spare respiratory capacity and glycolytic capacity

Thus far we showed that β2AR deficiency increases DC function, prompting us to elucidate the relevant mechanism. Recently, it has been shown that metabolism plays a crucial role in DC function (32). Glycolytic burst after LPS activation is signature for DC maturation and function (33). Therefore, we decided to address whether the β2AR KO DCs exhibit a metabolic profile similar to immunogenic DCs. We examined glycolysis and mitochondrial stress in WT and β2AR KO BMDCs at either 3 or 18 hours after LPS activation. In regards to mitochondrial stress, WT BMDCs had significantly higher spare respiratory capacity compared to β2AR KO BMDCs without difference in basal respiration and ATP production, indicating that β2AR DCs are more immunogenic DCs (Figure 3A). Same as previously reported (34), 18 hours after LPS activation, there was no response to FCCP and no difference between WT and β2AR KO BMDCs (Figure 3B). In regards to glycolysis, WT BMDCs had a higher glycolytic capacity compared to β2AR KO BMDCs at both 3 and 18 hours after LPS activation without any difference in glycolytic rate (Figure 3C and D respectively). Interestingly, the non-glycolytic acidification of β2AR KO BMDCs was significantly higher compared to WT BMDCs when no glucose was present in the media 3 hours after LPS activation yet without any difference at 18 hours after LPS activation. These data suggest that β2AR deficiency indeed changes the metabolic profiles of BMDCs and increases their immunogenicity.

## β2AR deficiency in BMDCs reduces STAT3 phosphorylation and improves the tumor-killing efficacy of $CD8^+$ T cells

STAT3 signaling plays an important role suppressing immunogenic function of DCs through increasing IL-10 secretion and inhibition of NF-κB (35, 36). This pathway was previously connected with β2AR signaling (37). Also, it has recently been shown that STAT3 expression in host myeloid cells inhibits GVHD severity through suppressing DC function (38). However, previous studies demonstrated that β2AR agonists activate STAT3 signaling pathway in cardiomyocytes and macrophages and suppress NF-κB (36, 37). In addition, mTOR signaling is also important for glycolysis in DC and increases commitment of cells to anabolism (39). Therefore, we assessed the phosphorylated form of STAT3 and mTOR in WT and β2AR KO BMDCS at different time points (Figure 4A). STAT3 phosphorylation is significantly decreased in β2AR KO BMDCs after LPS activation. There was no significant difference in mTOR phosphorylation after LPS activation, but β2AR KO BMDCs expressed more mTOR protein when normalized to β-actin internal control (Figure 4A).

To examine how β2AR affects the immunogenicity of DCs in allogeneic $CD8^+$ T cell response, we conducted MLR using WT or β2AR KO BMDCs with the addition of tumor cells. For MLR, WT and β2AR KO BMDCs were co-cultured with sorted $CD8^+$ T cells for 4 days. To assess tumor killing activity in the MLR, luciferase-expressing A20 tumor cells were added either at the beginning or day 3 of culture. Bioluminescence imaging was performed at day 4 to measure tumor burden in the MLR culture. Our control group consisted of only BMDCs and A20 cells. Regardless of when tumor cells were added at day 0 or day 3 (Figure 4B), there was significantly decreased tumor signal detected in the β2AR KO DCs compared to WT DCs. These data indicate that β2AR deficiency in BMDCs improves the tumor-killing efficacy of alloreactive $CD8^+$ T cells.

### β2AR deficiency in the hosts ameliorates GVHD and improves $CD4^+Foxp3^+$ regulatory T cell (Treg) reconstitution

To define the effect of β2AR KO in the host on GVHD, we used MHC-mismatched GVHD model where the host mice were injected with allogeneic TCD-BM and $CD8^+$ T cells. GVHD was measured using clinical GVHD score and body weight change (Figure 5A). As controls, WT and β2AR KO mice that received allogeneic TCD-BM only were able to recover and maintain their body weight and showed no sign of GVHD. In contrast, β2AR KO hosts that received TCD-BM and $CD8^+$ T cells showed less weight loss accompanied with less severe GVHD than WT hosts. Histopathological analyses of target organs showed higher pathological GVHD scores in the large and small intestines with a similar trend in the liver in WT hosts compared to β2AR KO hosts (Figure 5B).

To examine de novo T cell reconstitution in the hosts, we assessed donor-derived $CD4^+$ T cell absolute numbers and percentages at day 35 and 60 in the spleen. β2AR KO mice have higher numbers of reconstituted $CD3^+CD4^+$ T cells (Supplemental Figure 1C). Because of the importance of reconstituted Tregs in controlling GVHD, we assessed Treg percentage and absolute number. There was no significant difference between WT and β2AR KO hosts regarding $CD4^+Foxp3^+$ Treg percentages at day 35, but Treg cell absolute numbers were noticeably higher in β2AR KO hosts (Figure 5D). At day 60, both Treg percentages and absolute numbers were significantly higher in β2AR KO hosts compared to WT hosts (Figure 5C and D). These results indicate that β2AR deficiency in the host induces not only higher expansion of transplanted donor $CD8^+$ T cells (Supplemental Figure 1A), but also improved reconstitution of $CD4^+$ T cells including Tregs from donor BM-derived progenitors and stem cells.

## Discussion

Host APCs are a vital parameter in GVT and GVHD pathogenesis. In this study, we discovered that β2AR deficiency in the host increases GVT effect. We also showed that β2AR blockade with a selective blocker before BM transplantation resulted in significantly enhanced GVT effect. Our data demonstrated that β2AR plays a suppressive role on DC function via decreasing expression of co-stimulatory markers and increased IL-10 cytokine release. In agreement with our report, it has been shown that β2AR agonists such as isoprenaline and salbutamol suppress inflammatory phenotype of DCs (23, 24). Also our

data showed that the increased immunogenic phenotype of β2AR-deficient DCs is associated with downregulation of STAT3 phosphorylation. β2AR signaling was previously connected with STAT3 activation (37), which led to increased IL-10 secretion and inhibition of NF-κB (35, 36). Also, it has recently been shown that STAT3 expression in host myeloid cells inhibits GVHD severity through suppressing DC function (38). Through MLR-based *in vitro* T cell activation and tumor cell killing experiments, we demonstrated that β2AR deficiency in DCs enhances alloreactive $CD8^+$ T cell response in the tumor setting. However, our previous study showed that β2AR inhibition exacerbated GVHD induced by total T cells in which $CD4^+$ T cells presumably play a dominant role. Put together, these findings suggest that β2AR signaling plays more complicated and differential roles in GVT and GVHD involving $CD4^+$ versus $CD8^+$ T cell responses and possibly other immune cells. Further study will be required to delineate the underlying mechanisms.

We show that β2AR deficiency in DCs decreases spare respiratory capacity and glycolytic capacity. Also β2AR KO DCs use glutamine as a source of energy more than WT DCs with a negative trend in glycolytic rate which is in agreement with previous reports indicating that high glucose can also suppress DC function marked by increased IL-10 secretion and down-regulation of costimulatory markers (40) and represses DC-induced T cell responses (41). Metabolic changes through mTOR signaling play a critical role in DC functions and cytokine release. High glycolysis with active mTOR signaling and less OXPHOS activation is a signature of activated DCs (39). In tolerogenic DCs, OXPHOS activation plays a major role in DC metabolism and energy generation through inhibition of mTOR signaling and activation of AMPK signaling (42). In line with previous reports, we showed that β2AR KO DCs express more mTOR proteins compared to WT DCs. It has also been shown that high mitochondrial respiration and glycolytic capacity is the phenotype of tolerogenic DCs (43), which is in agreement to what we found in WT DCs compared to β2AR KO DCs. Altogether, for the first time to our knowledge, our data confirm that β2AR deficiency shifts DC metabolism towards a more immunogenic phenotype.

It has been documented that an increased percentage of T cells including regulatory T cells (Tregs) after transplantation is a sign of improved reconstitution (44). After allogeneic transplantation, reconstituted Tregs play a crucial role in the control of DC co-stimulation and GVHD without affecting T cell expansion (45, 46). It has also been shown that occurrence of GVHD impairs immune cell reconstitution (47). Interestingly, the enhanced Treg reconstitution at day 60 in the β2AR KO hosts is associated with increased $CD8^+$ T cell number and memory phenotype (Supplemental Figure 1A–B) and deceased GVHD. This result is also consistent with a previous report showing that Tregs promote memory $CD8^+$ T cell maturation (48).

Finally, we summarized our finding in Supplemental Figure 2. In conclusion, our data demonstrate that β2AR signaling suppresses host DC function. Either blockade or knockout of host β2AR enhances GVT effect through modulating STAT3 and mTOR signaling in host DCs, resulting in up-regulation of co-stimulatory molecules and more immunogenic DCs. In addition, β2AR deficiency in the hosts induces enhanced T cell reconstitution and memory formation, which may translate into increased GVT activity without exacerbating GVHD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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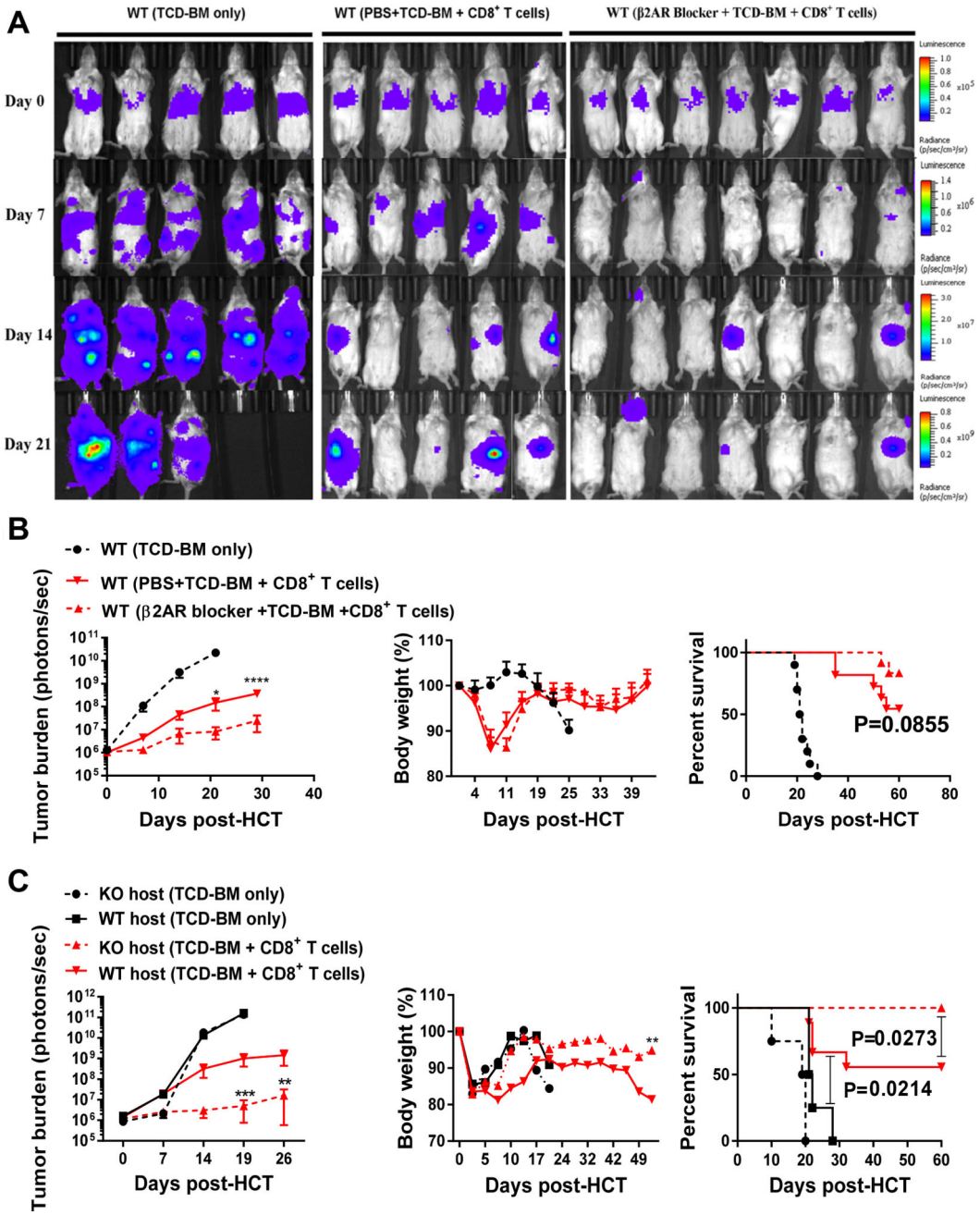


**Figure-1. Blockade of β2AR in the recipients improves GVT effect**

BALB/cJ host mice were pretreated with β2AR blocker for 7 days before transplantation. At day −1, host mice were irradiated with 900 cGy and at day 0 mice were transplanted with $3\times10^6$ TCD-BM and $3\times10^5$ $CD8^+$ T cells purified from C57BL/6J donor mice after IV injection of $1\times10^5$ luciferase-expressing A20 cells. Bioluminescence imaging was performed to measure tumor burden. (A) Representative images of tumor burden, and (B) summary data of tumor burden, body weight and survival rate were presented (n=10–13 for each group). (C) At day −1, BALB/cJ WT and β2AR KO mice were irradiated with 900 cGy and at day 0 were transplanted with $3\times10^6$ TCD-BM and $3\times10^5$ $CD8^+$ T cells purified from

C57BL/6J donor mice after IV injection of $1\times10^5$ A20 cells (*n=10*). Data are presented as mean ± SEM from two replicate experiments. **** P < 0.0001; *** P < 0.001; ** P < 0.01; * P < 0.05.

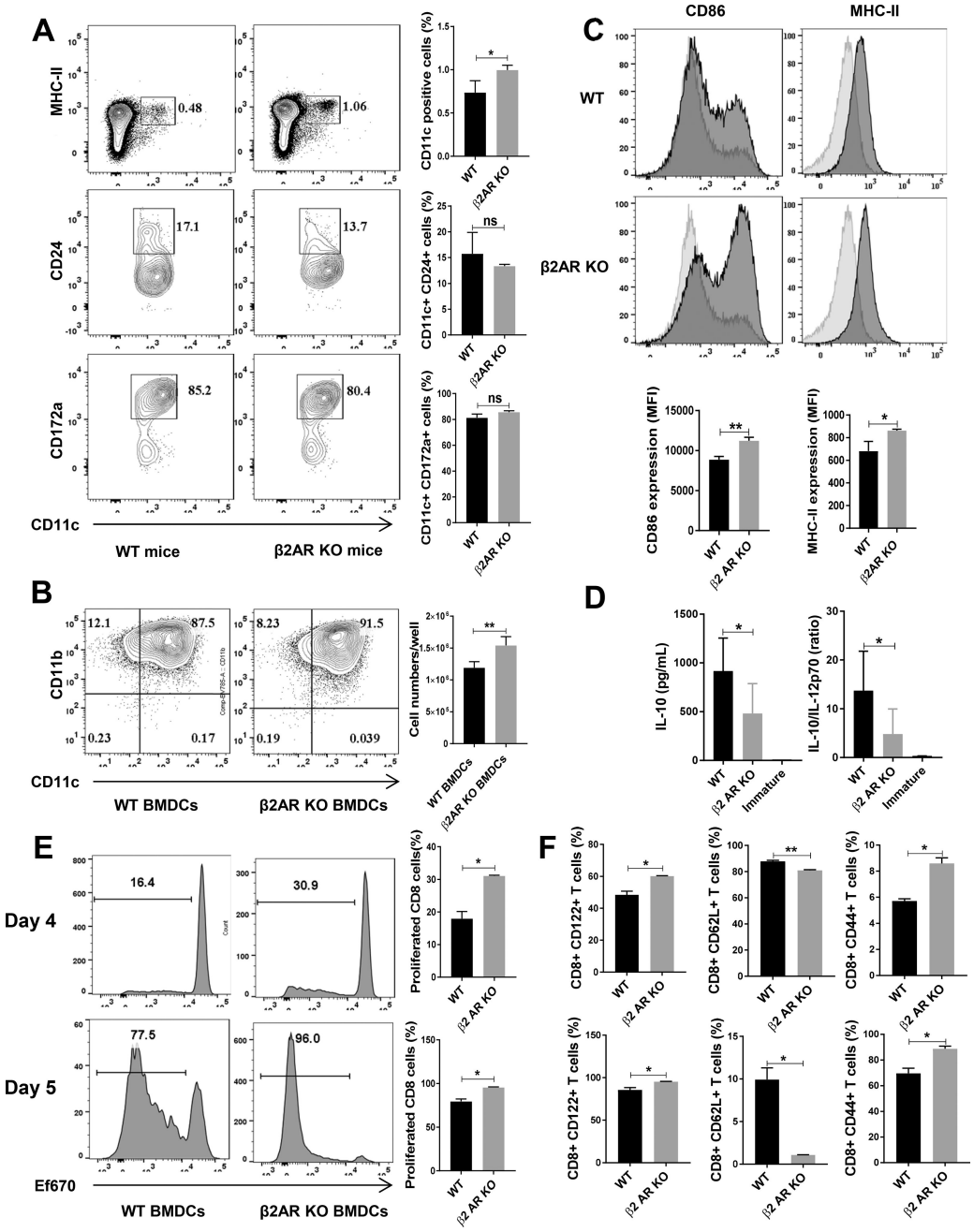


**Figure-2. β2AR suppresses DC proliferation and function**

(A) Representative flow cytometry plots of DC percentages and subpopulations in spleens of naïve WT and β2AR KO mice. The top two plots were gated on live splenocytes and the CD24 and CD172a plots were gated on live $CD11c^+$ cells. Summary data are pooled from 2 repeated experiments with 3 mice in each group. (B) Flow cytometry plots of BMDCs developed from WT and β2AR KO mice as described in the Methods. Summary data are from 2 repeated experiments with 6 samples in each group. (C) Representative flow cytometry plots of CD86 and MHC-II expression in WT and β2AR KO BMDCs. Summary data are from 3 repeated experiments with 3 samples in each group. (D) Cytokine production

by WT or β2AR KO BMDCs. Summary data are from 2 repeated experiments with 6 samples in each group. (E) The proliferation of $CD8^+$ T cells, quantified by using ef670 proliferation dye, in MLR stimulated by WT or β2AR KO BMDCs for 4 and 5 days. Summary data are from 3 repeated experiments with 3 samples in each group. (F) Expression of CD122 as an activation marker, and effector phenotype markers CD44 and CD62L on $CD8^+$ T cells in MLR stimulated by WT or β2AR KO BMDCs for 4 and 5 days. Summary data are from 3 repeated experiments with 3 samples in each group. ** P <0.01; *P <.05.

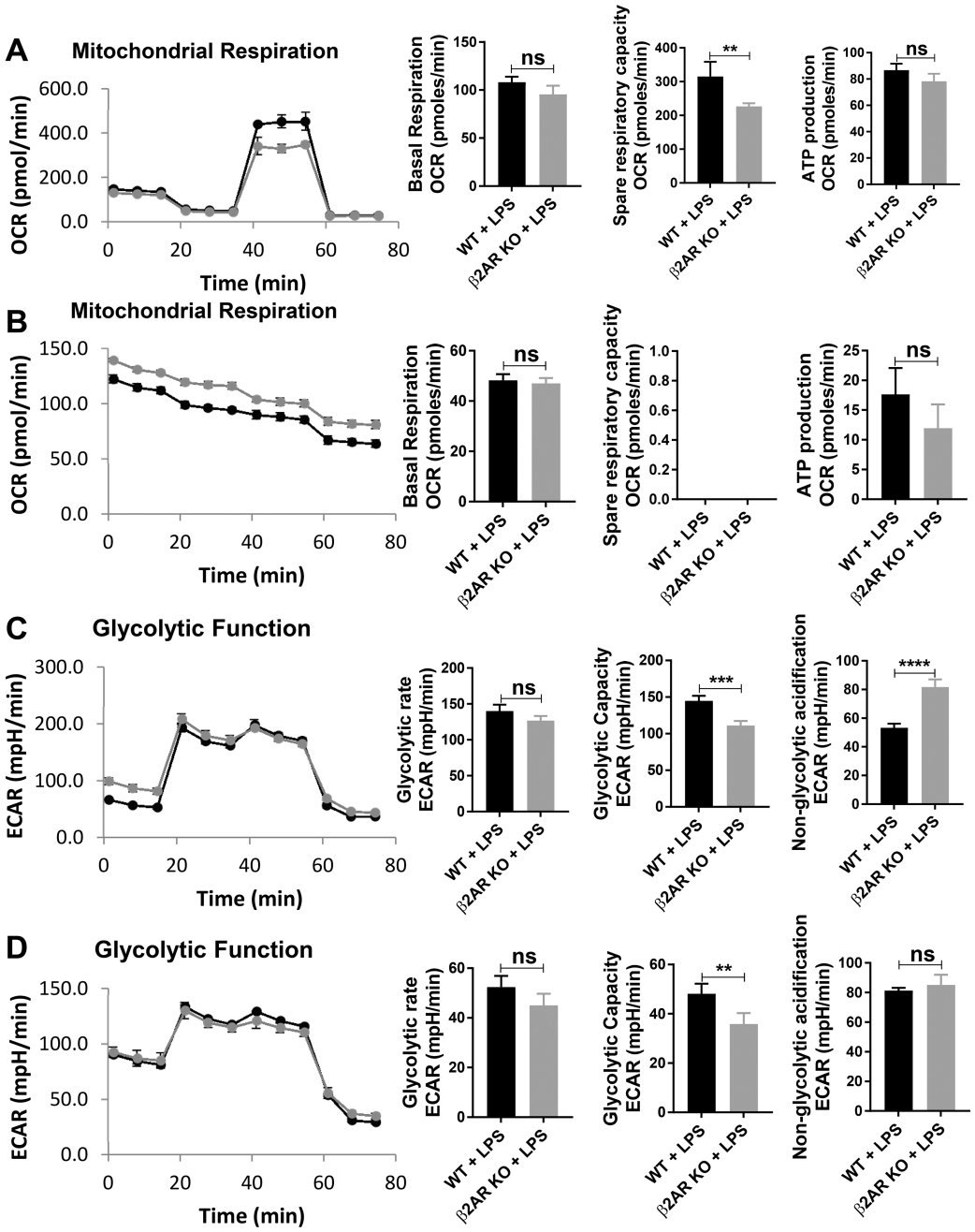


**Figure-3. β2AR deficiency in BMDCs decreases spare respiratory capacity and glycolytic capacity without changing ATP production and glycolytic rate**

(A and B) Representative kinetic study of mitochondria OCR (mpH/min) in WT (Blue) and β2AR KO (Red) BMDCs after adding LPS for (A) 3 hours and (B) 18 hours by using sequential addition of oligomycin (Olig), FCCP, and rotenone/antimycin A (Rot-AA). Summary bar graphs show basal respiration, spare respiratory capacity and ATP production in WT and β2AR KO BMDCs. OCR for spare respiratory capacity was zero because DCs did not response to FCCP 18 hours after LPS activation. (C and D) Representative kinetic study of glycolysis-dependent ECAR (mpH/min) in WT (Blue) and β2AR KO (Red) BMDCs, after adding LPS for (C) 3 hours and (D) 18 hours by using sequential addition of

glucose (Gluc), oligomycin (Olig), and 2-DG. Summary bar graphs show glycolytic rate, glycolytic capacity, and non-glycolytic acidification in WT and β2AR KO BMDCs. Data were pooled from three independent experiments and presented as Mean ± SD. **** $P < 0.0001$; *** $P < 0.001$; ** $P < 0.01$.

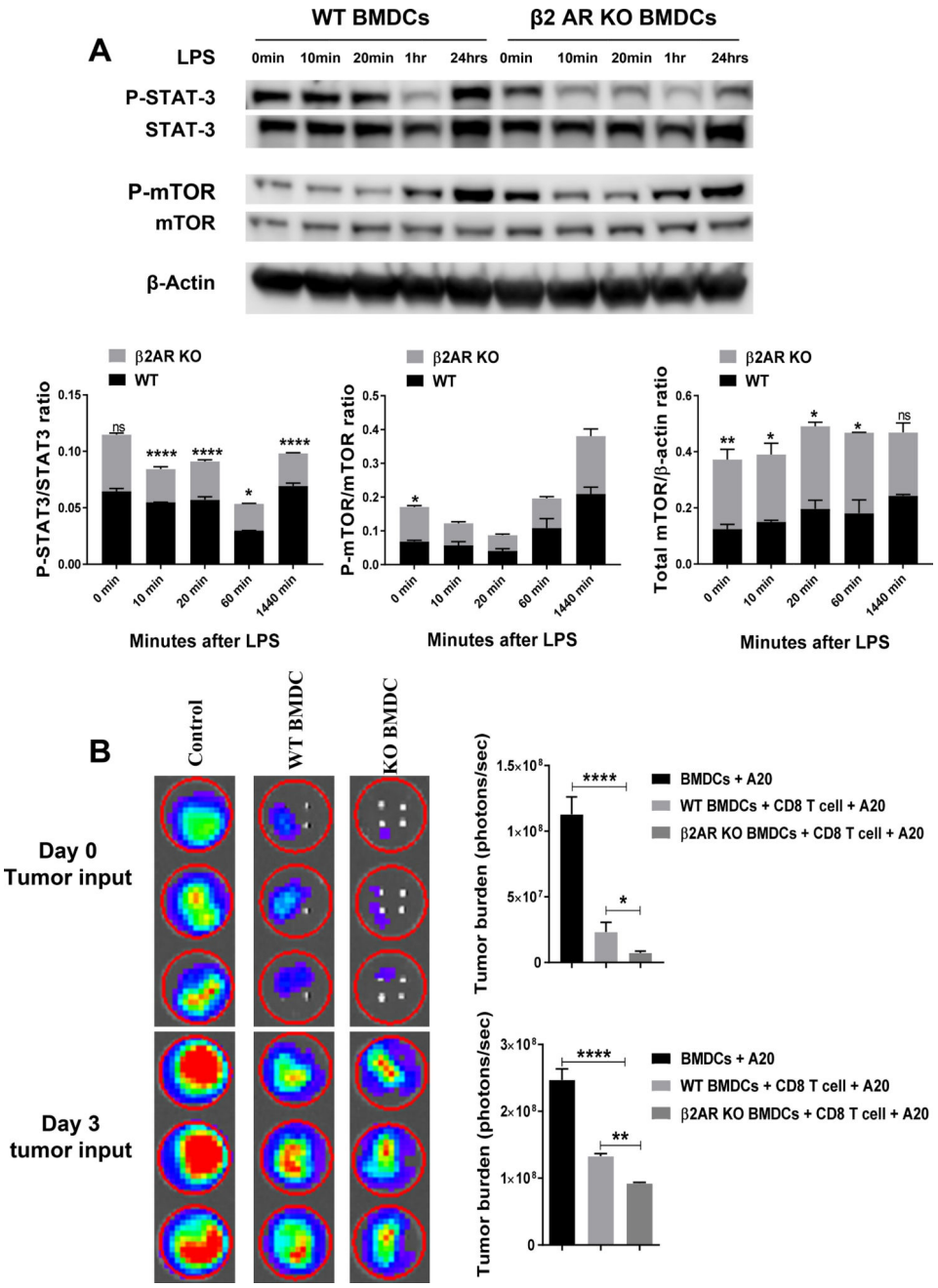


**Figure-4. β2AR deficiency in BMDCs modulates STAT3 and mTOR activity and increases CD8$^+$ T cell-mediated tumor killing**

(A) STAT3 and mTOR phosphorylation in WT and β2AR KO BMDCs after LPS activation. Representative western blots of P-STAT3 and P-mTOR proteins and quantification of P-STAT3/STAT3, P-mTOR/mTOR and total mTOR/β-actin ratios (n=3). Data are presented as mean ± SD. ****, P < 0.0001; ***, P = 0.001; **, 0.01; *, 0.05. (B) Mature BMDCs were generated from BALB/cJ WT and β2AR KO mice and co-cultured for MLR with CD8$^+$ T cells purified from C57BL/6J mice in a ratio 1:5. At day 0 or day 3 of MLR, A20 tumor cells were added to MLR. Bioluminescence imaging was performed at day 4 to measure

tumor burden in each well. Data are presented as mean ± SD. **** P < 0.0001; ** P <0.01; *P <0.05.

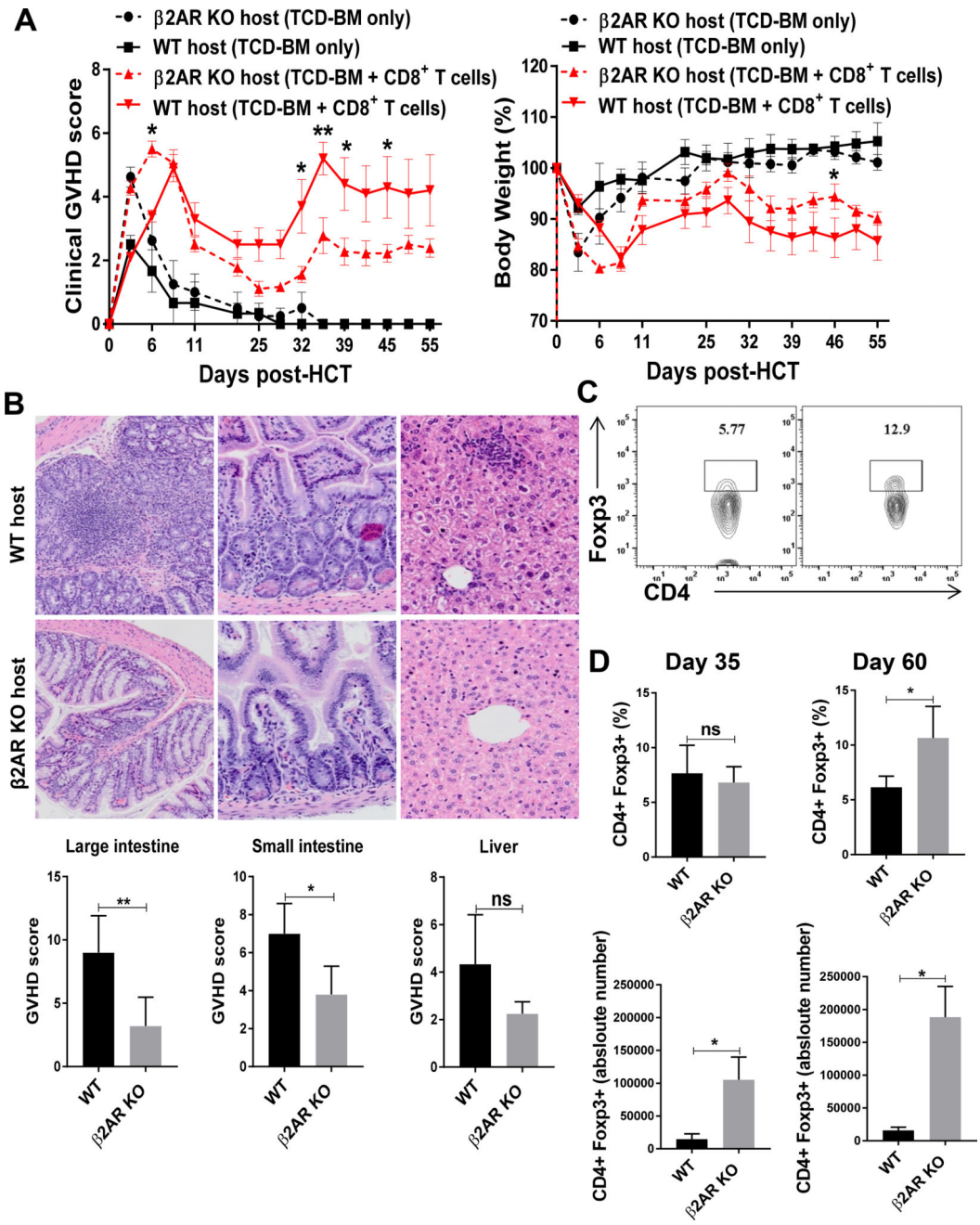


**Figure 5. β2AR deficiency in the host decreases GVHD and improve Treg reconstitution**

At day −1, BALB/cJ WT or β2AR KO host mice were irradiated with 900 cGy. At day 0, host mice were transplanted with $3\times10^6$ TCD-BM and $2\times10^6$ $CD8^+$ T cells purified from C57BL/6J mice. (A) Clinical GVHD score and body weight (*n=17*). (B) Representative histopathological images shown at 200× magnification, with pathological GVHD scores in the large intestine, small intestine and liver (*n=5*). (C) Representative flow cytometry plots for Tregs at day 60. (D) Percentages and absolute numbers of Tregs in the host spleens (*n=8*). Data are presented as Mean ± SD. ****, P < 0.0001; ***, P = 0.001; **, 0.01; *, 0.05.