4-Octyl itaconate reverses immune hyperactivation of T cells in aGVHD by inhibiting GAPDH

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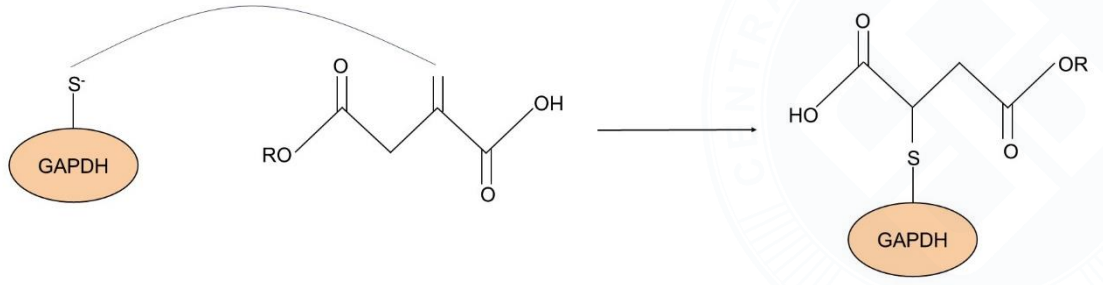
Backgrounds

Classically activated effector lymphocytes require glycolysis for survival, differentiation, and effector functions. Activated T cells switch from OXPHOS to aerobic glycolysis, presenting a potential therapeutic target in immune over activation disease such as aGVHD.

4-OI is a cell-permeable derivative of endogenous itaconate, has been implicated in immunoregulation, oxidative stress, and lipid peroxidation.

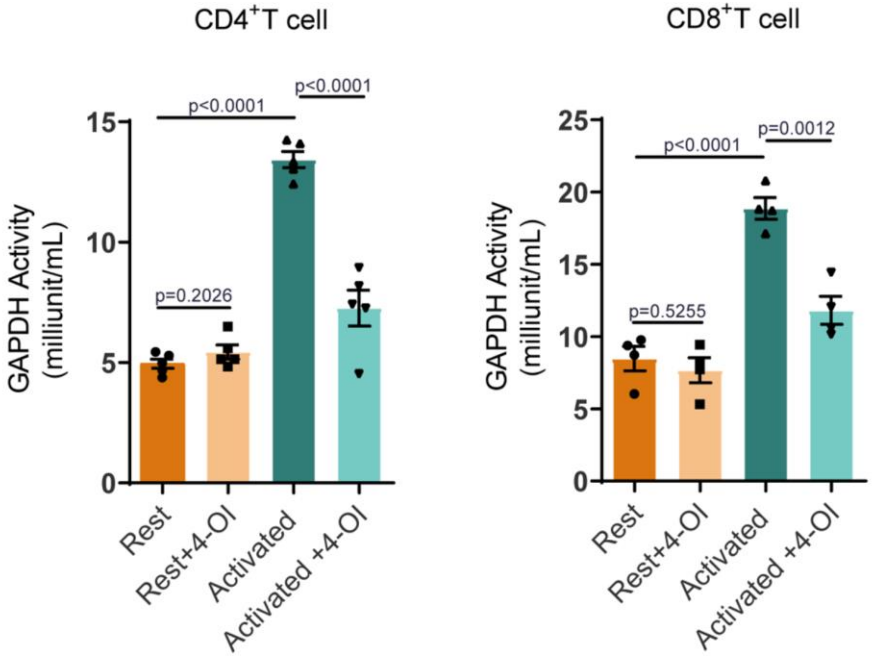
It can suppress the inflammatory response in macrophages by targeting GAPDH.

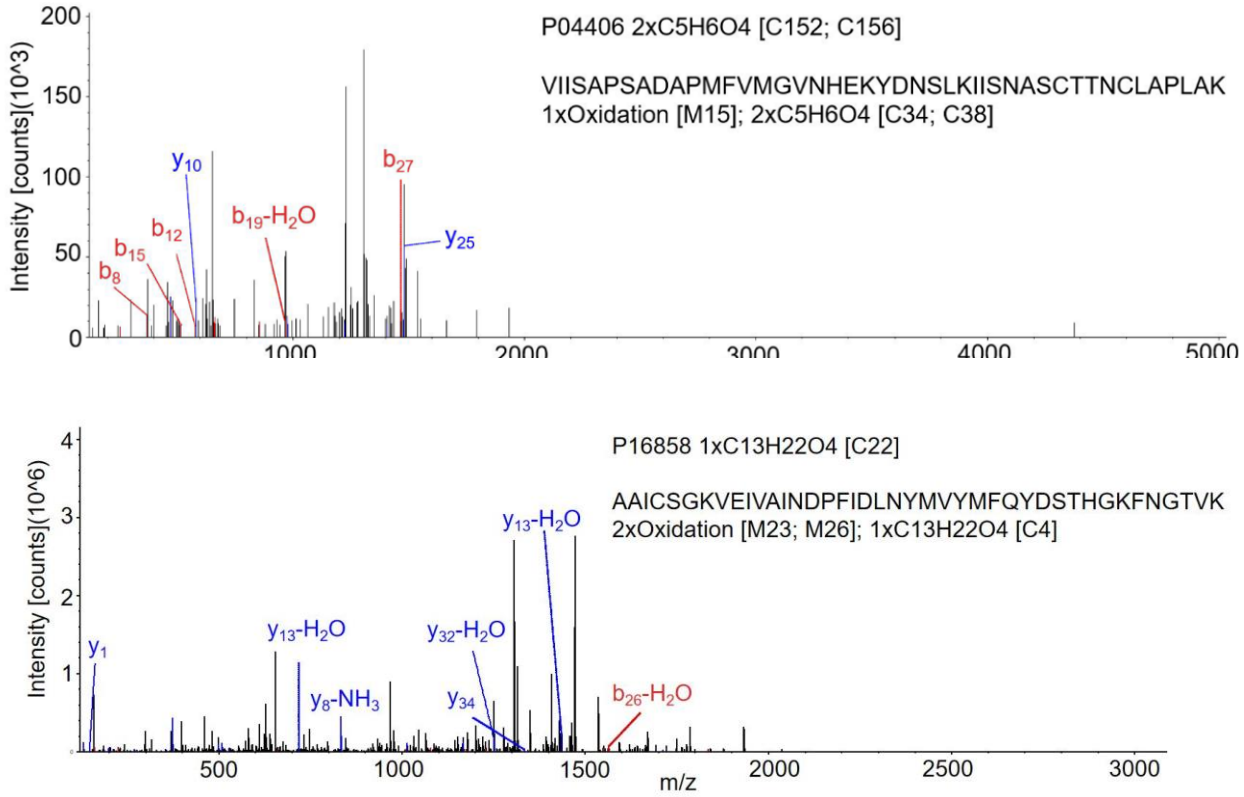
However, whether 4-OI could reverse the metabolic reprogramming of stimulated lymphocyte in aGVHD by partial suppression of GAPDH has not been explored.



In vitro results

4-OI inhibit the GAPDH activity in activated CD4+ and CD8+ T cells by alkylated modification



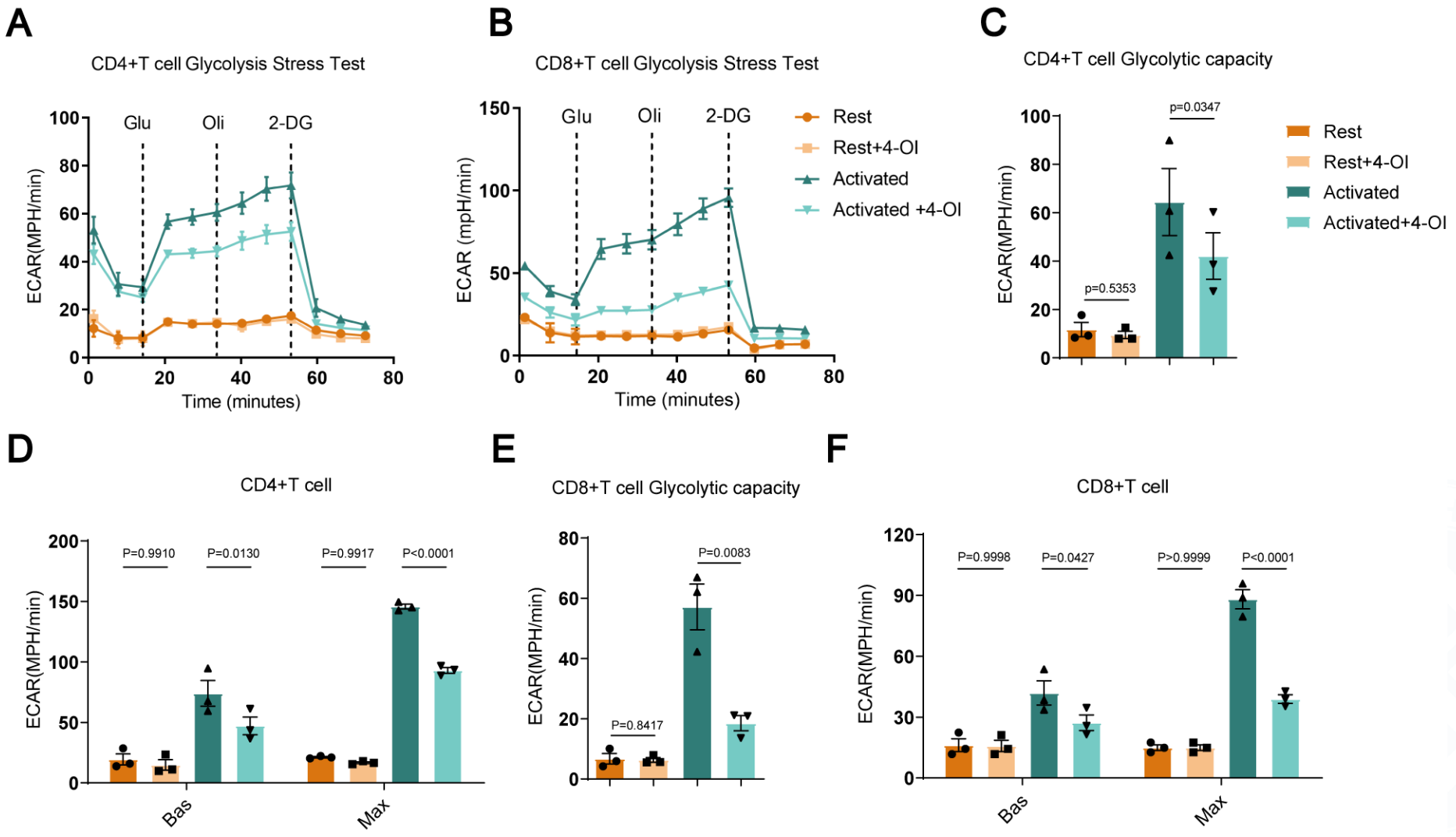


4-OI does not affect GAPDH activity in resting CD4+ and CD8+ T cells but the activated T cells.

LC-MS/MS showed that in human activated T cells treated with 4-OI, Cys 152 and Cys 156 of GAPDH was alkylated by itaconate. And in mouse activated T cells， Cys 22 of GAPDH was alkylated by 4-OI.

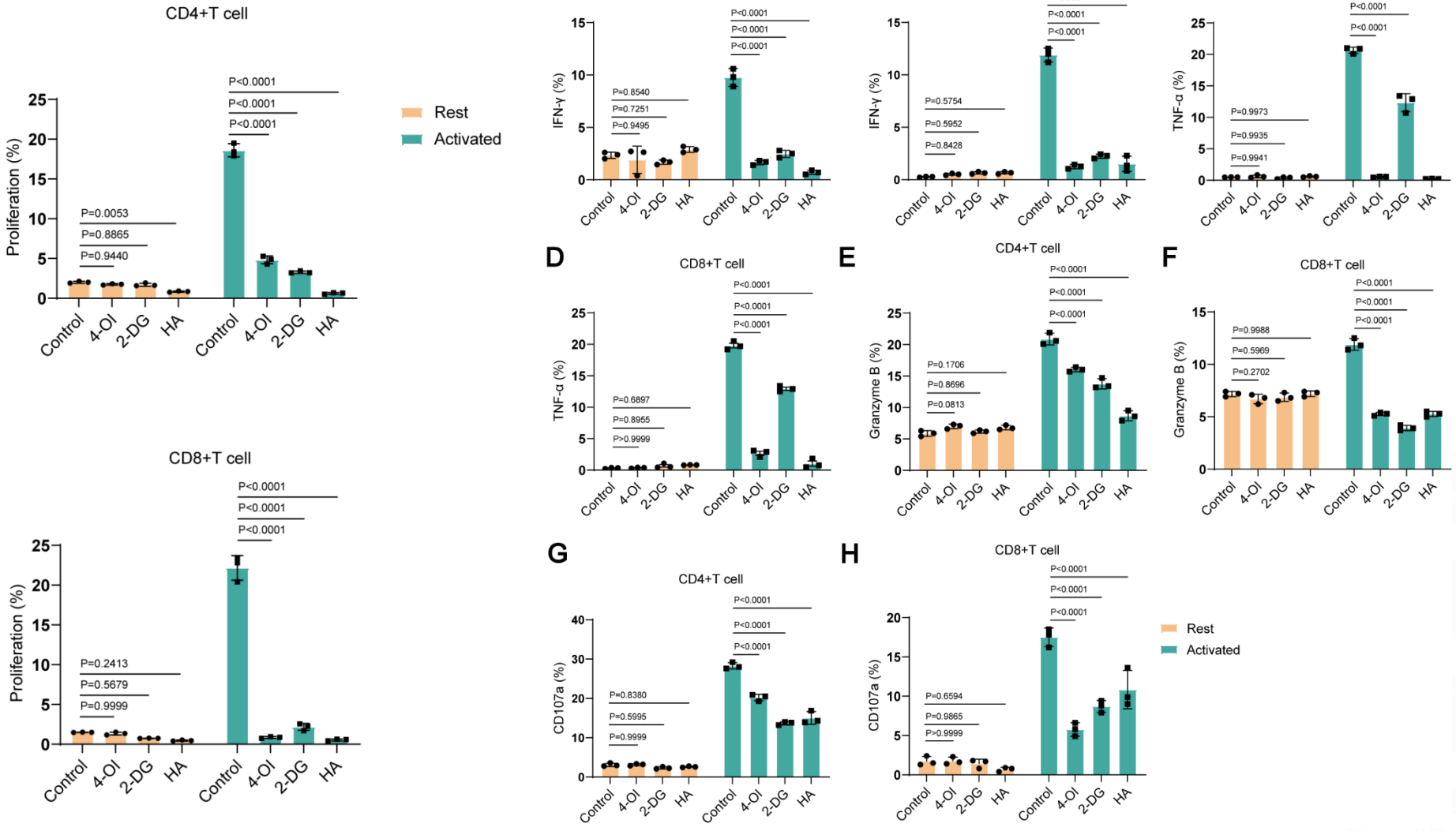
In vitro results

GAPDH inactivation by 4-OI inhibits glycolysis in activated T cell



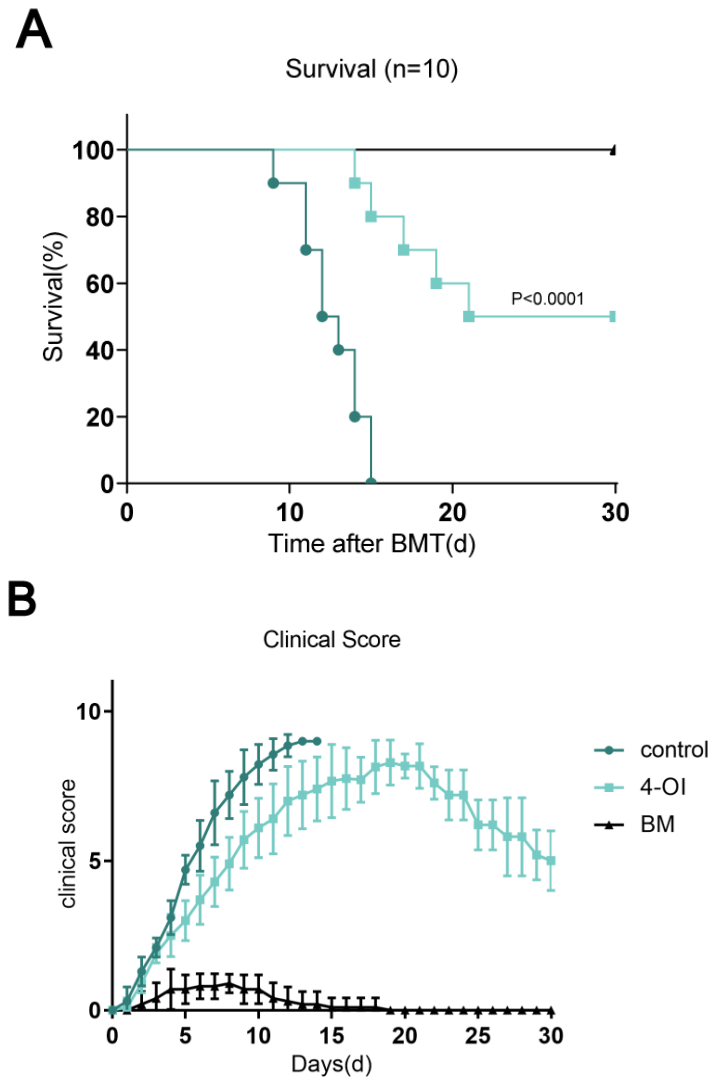
In vitro results

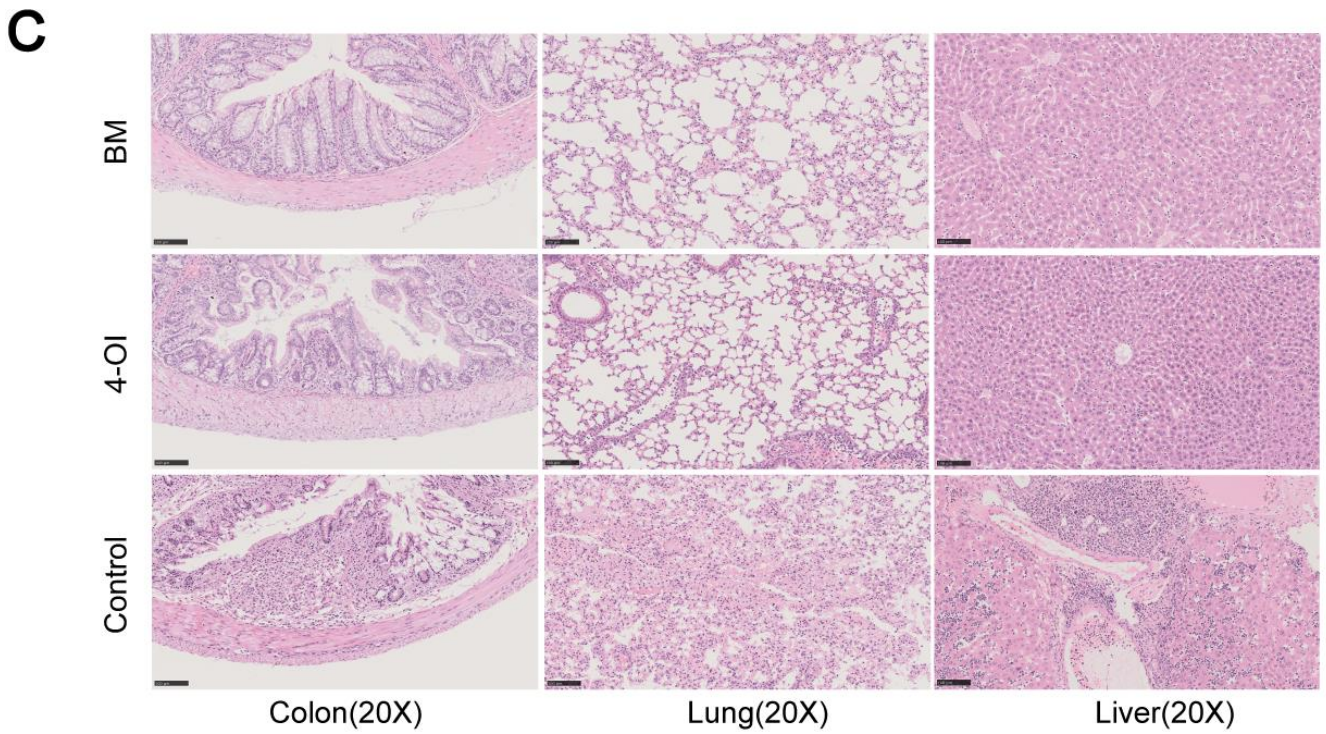
4-OI regulates the proliferation and function of activated T cells



In vivo results

4-OI treatment reduces T cell–mediated GVHD in allo-HSCT

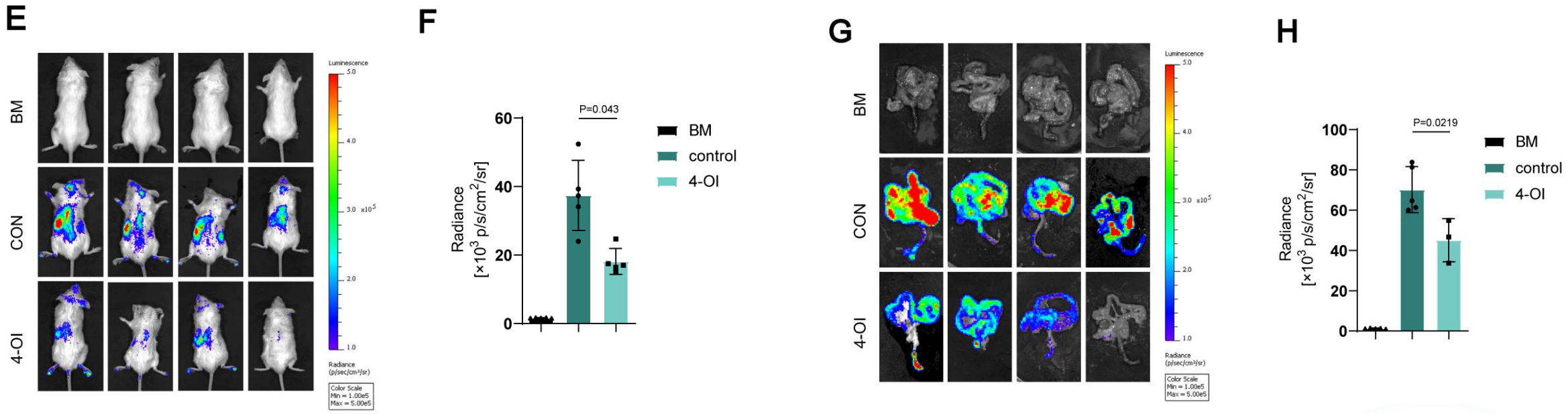




We established the aGVHD model of mouse to assess the function of 4-OI in GVHD following allo-HSCT. 4-OI treatment significantly increased the survival rate of mice with GVHD following allo-HSCT, had significantly fewer pathological injuries and significantly lower GVHD clinical score, compared to the control group.

In vivo results

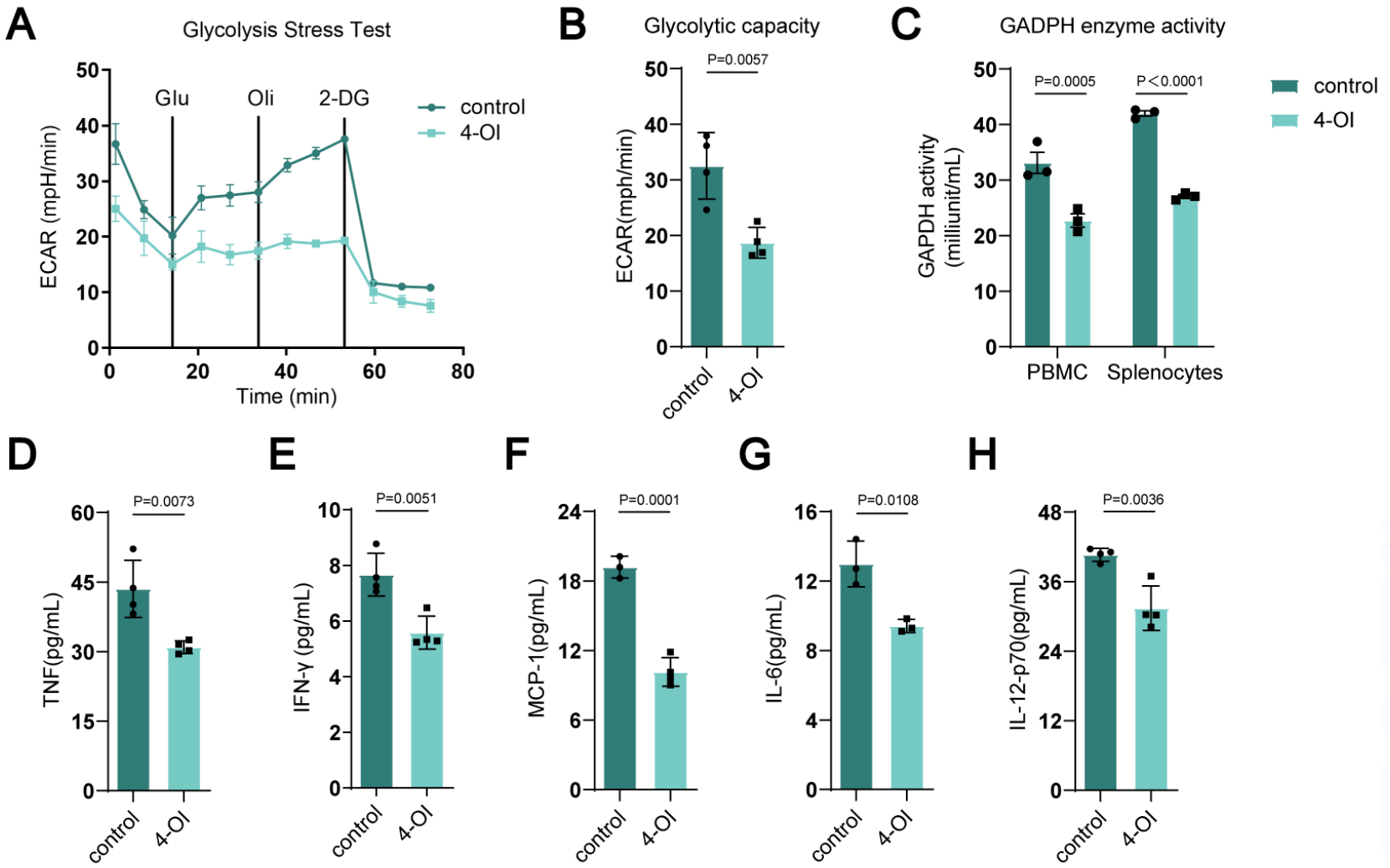
4-OI reduces the proliferation of donor T cells in vivo



The activation of allogeneic donor T lymphocytes causes the tissue damage associated with GVHD. We used L2G85 mice（C57BL/6 Background）donor T cells that express luciferase linked to the β-actin promoter. 10 days after allo-HSCT, Balb/c recipients were subjected to bioluminescence to measure donor T cell expansion. Quantification of whole-body or intestinal BLI intensity indicated that 4-OI had significantly reduced T cell expansion in recipient, as compared to that of vehicle recipients. Therefore, 4-OI can reduce the proliferation of T cells induced by allogeneic antigen response in vivo.

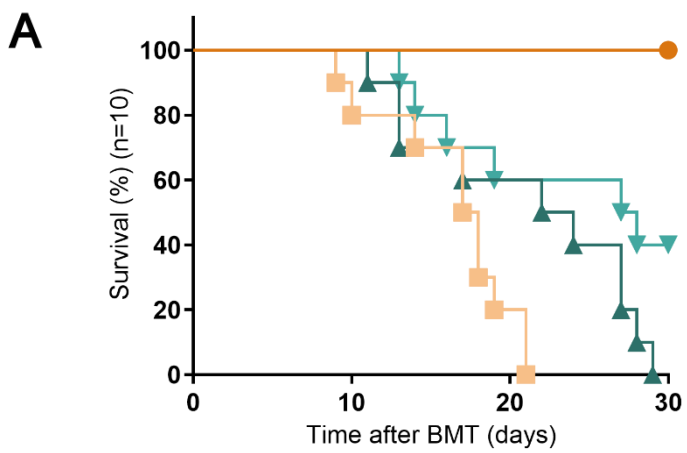
In vivo results

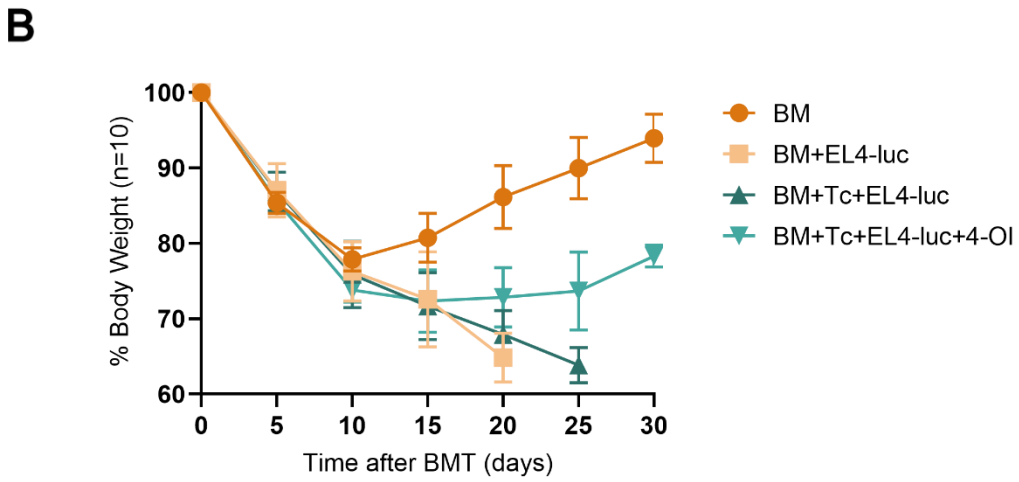
4-OI inhibits inflammation and downregulates aerobic glycolysis

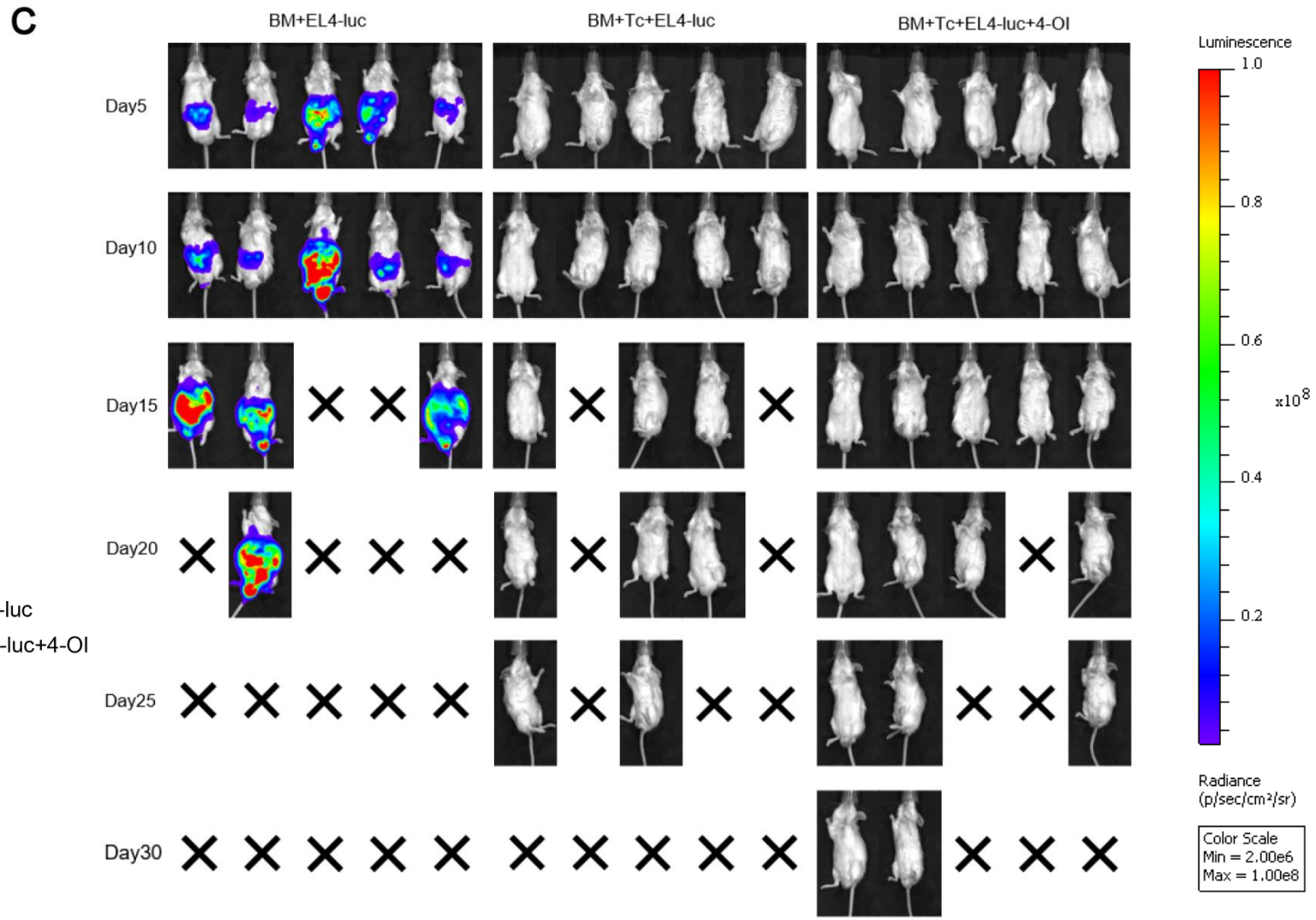


In vivo results

4-OI treatment maintained GVL activity.







Conclusion

These data indicated that 4-OI could inhibit the proliferation and function of over activated T cells by reversing metabolic reprogramming which suggested 4-OI a potential therapeutic agent in GVHD and may be other autoimmune diseases.

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