

Appendix 1: Using immunomagnetic bead positive sorting technology to extract and sort mouse spleen-derived CD4⁺ T cells

- 1) The BALB/c mice was quickly put to death use the cervical dislocation method, and then put most of them in the pre-prepared 75% alcohol to soak and disinfect for 5 minutes;
- 2) Take out the spleen aseptically and place it in a pre-prepared 15ml centrifuge tube containing an appropriate amount of RPMI-1640 medium on ice;
- 3) Shake gently to wash away the remaining blood and tissue residues on the surface of the fresh spleen for grinding;
- 4) Under dark conditions, put 4.5ml of lymphocyte separation solution in a 60 mm petri dish, cover the culture dish with a sterile 200 mesh nylon mesh and fully contact the lymphocyte separation solution;
- 5) Put the spleen in the center of the nylon membrane, Grind the piston vertically and evenly for about 3 minutes with moderate force, and shake it gently to disperse the cell clusters into the separation liquid;
- 6) After fully grinding, collect the cell suspension and transfer it to a sterile 15ml centrifuge tube, then draw 200ul of 1640 medium slowly into the centrifuge tube along the wall of the tube, keep the liquid level clear, and centrifuge for 30min at 20°C and 800×g;
- 7) After the centrifugation, aspirate the middle cell layer into a dry and sterile 15ml centrifuge tube, then add 10ml 1640 medium and mix upside down, wash twice, and centrifuge again at 20°C and 250×g for 10 minutes.
- 8) Discard the supernatant, add 3-5ml 1640 medium to resuspend, after fully dissipating the cell mass, take 20ul cell suspension and add it to the blood cell counting plate to determine the number of mouse splenic mononuclear cells;
- 9) Counting formula: the total number of mononuclear cells = [(the total number of cells in the 4 large cells on the counting plate)/4] × the dilution factor × 10⁴/ml), and the cell viability is determined at the same time: the cells are suspended by the trypan blue exclusion method.
- 10) Mix the solution with 0.4% trypan blue staining solution 1:1 and count under a microscope. A total of 200 cells are counted within 5 minutes. Survival rate=number of live cells/total number of cells×100%.
- 11) The obtained spleen mononuclear cell suspension (counting completed) was washed once with ice PBS, centrifuged at 4°C and 300×g for 10 minutes, and the supernatant was discarded for sorting; Next, the magnetic bead sorter and the operating table were prepared.
- 12) The cells were resuspended at the ratio of 90ul magnetic bead sorting buffer per 10⁷ cells; immunomagnetic bead positive sorting technology was used to positively sort mouse CD4⁺ T cell subsets.

Table S1 Primers for CNR2, STAT5, ROR γ t and FOXP3

Gene	Primer sequences (forward)	Primer sequences (reverse)
<i>CNR2</i>	ATGGCCGTGCTCTATATTATCCT	ATGGTCACACTGCCGATCTTC
<i>STAT5</i>	CAGCCGTGGGATGCTATTGA	GGGACAGCGGTCATACGTG
<i>RORγt</i>	GACCCACACCTCACAAATTGA	AGTAGGCCACATTACACTGCT
<i>FOXP3</i>	CACCTATGCCACCCTTATCCG	CATGCGAGTAAACCAATGGTAGA
<i>GAPDH</i>	TGTCGTCATGGGTGTGAAC	ATGGCATGGACTGTGGTCAT