Supplemental material

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**Figure S1.** Over-expression MKL-1 and STAT5b increase the number of Treg in CD3+ T cells and enhance the Treg markers expression.

**A**. Western blot analysis of MKL-1 and STAT5b protein level in CD3+T cells transfected with myc-MKL-1 or flag-STAT5b for 48 hours.

**B**.The number of Treg in CD3+T cells transfected with myc-MKL-1 or flag-STAT5b for 48 hours by flow cytometry.

**C**. QPCR analysis of Foxp3 and CD25 mRNA level in CD3+T cells transfected with myc-MKL-1 or flag-STAT5b for 48 hours. GAPDH is the loading control. \*\*, *P*<0.01,\*, *P*<0.05. n=3.

**D** and **E.** Western blot analysis of Foxp3 and CD25 protein level in CD3+T cells transfected with myc-MKL-1 or flag-STAT5b for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01,\*, *P*<0.05. n=3.

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**Figure S2.** Inhibited or knock-down MKL-1 and STAT5b weaken the Treg markers expression.

A. QPCR analysis of Foxp3 and CD25 mRNA level in CD3+T cells treated with AG490 or Y27632 for 48 hours. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

B. QPCR analysis of Foxp3 and CD25 mRNA level in CD3+T cells transfected with MKL-1 and STAT5b siRNA for 48 hours. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

C and E. Western blot analysis of Foxp3 and CD25 mRNA level in CD3+T cells treated with AG490 or Y27632 for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

D and F. Western blot analysis of Foxp3 and CD25 protein level in CD3+T cells transfected with MKL-1 and STAT5b siRNA for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

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**Figure S3.** IL2 affects the effect MKL-1 and STAT5b on the Treg marker expression.

A. QPCR analysis of Foxp3 protein level in CD3+T cells transfected with MKL-1 and STAT5b and treated with IL2 for 48 hours. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05.n=3.

B and C. Western blot analysis of Foxp3 protein level in CD3+T cells transfected with MKL-1 and STAT5b and treated with IL2 for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

D. The luciferase reporter assays were used to test the transactivity of Foxp3 in CD3+T cells transfected with MKL-1 and STAT5b and treated with IL2 for 48 hours. \*\*, *P*<0.01, \*, *P*<0.05, n=6.

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**Figure S4.** Ag490 and Y27632 affect the phosphorylation of Foxp3 and nuclear accumulation of Foxp3.

A and B. Western blot analysis to detect phosphorylated Foxp3 in CD3+T cells treated with AG490 or Y27632 for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.

C and D. Western blot analysis to detect nuclear or membrane Foxp3 in CD3+T cells treated with AG490 or Y27632 for 48 hours. Data were quantified using Quantity One software. GAPDH is the loading control. \*\*, *P*<0.01, \*, *P*<0.05. n=3.