

Materials and methods

Mice

The *Foxp3*^{YFP-Cre} mice (Jax No. 016959) (Rubtsov *et al.*, 2008) were gifts from Prof. Xiaoming Feng (Wu *et al.*, 2017). The *Bim-flox* mice (Strain No. T007185) were purchased from GemPharmatech (Nanjing, China). All mice were maintained in specific pathogen free (SPF) conditions.

Flow cytometry

For analysis of cell surface markers, cells were washed in PBS containing 2% (v/v) FBS and stained with indicated antibodies, intracellular proteins were stained with Pharmingen Transcription Factor Buffer Set (BD Pharmingen, 562574), followed with flow cytometry analysis (Beckman, CytoFLEX LX).

Antibodies used in this research: anti-CD4 (GK1.5, eBioscience), anti-CD8 α (53-6.7, BD Pharmingen), anti-CD44 (IM7, Biolegend), anti-CD62L (MEL-14, Biolegend), anti-Foxp3 (150D, Biolegend), anti-Bim (C34C5, Cell Signal Technology).

Transcriptome profiling

CD4⁺YFP⁺ Treg cells were sorted from the peripheral lymph nodes and spleens of *Foxp3*^{Cre} control mice (8-10-weeks-old), *Foxp3*^{Cre};*Bim*^{fl/fl} mice (8-10-weeks-old), or *Foxp3*^{Cre} mice (older than 18-months-old), respectively. Detailly, peripheral lymph nodes and spleens were harvested from mice, and grinded into single cells, then the CD4⁺-T cells were isolated by Mouse CD4 T Lymphocyte Enrichment Set-DM (BD Biosciences, 558131), followed with FACS sorting (SONY Cell Sorter, SH800S) to

purify CD4⁺YFP⁺ Treg cells with purities >99 %.

RNA samples were prepared with the miRNeasy Mini Kit (Qiagen, 21704), then reverse transcribed, amplified, labeled (Affymetrix GeneChip pico kit, 703308), and hybridized to Clariom D Arrays, mouse (Affymetrix, 902931). Microarray data sets were analyzed with Applied Biosystems Expression Console Software 1.4.

***in situ* genome editing of hepatocytes**

The SB100X in pCAG globin pA plasmid (Addgene plasmid No. 127909) was gift from Prof. Mark Groudine. The pT3-myr-AKT-HA and pT3-EF1a-NICD1 plasmids (Addgene plasmid No. 31789 and 46047, respectively) were gifts from Prof. Xin Chen (Fan *et al.*, 2012). 20 mg NICD, 4 µg AKT, and 1 µg SB100 transposase plasmids were diluted in 2 ml 0.9 % NaCl, and injected into a lateral tail vein within 5-7 s.

Statistical analysis

The *p* values were calculated by Mann-Whitney test, two-tailed unpaired Student's t-test, one-way ANOVA or two-way ANOVA as indicated using GraphPad Prism, unless otherwise noted. Statistical analysis of mouse survival and respective *p* values were determined using the log-rank test. *p* < 0.05 was considered as significant. All error bars represent the s.e.m. from three independent experiments.

References

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