

Fig.S1

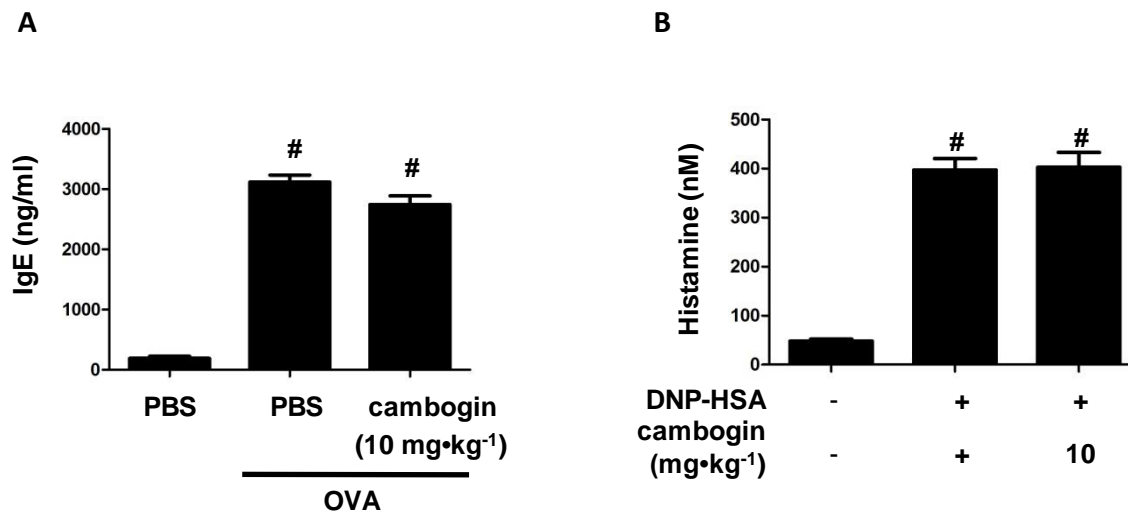


Fig.S1. (A) Seven-week-old female Balb/c mice were sensitized on days 0 and 14 by intraperitoneal (i.p.) injection with 20 μ g of OVA (Sigma-Aldrich, St. Louis, MO) in PBS mixed with equal volumes of alum as an adjuvant in a total volume of 200 μ L. On days 22, 23 and 24, the mice were exposed to aerosolized OVA (1% OVA in PBS) or PBS for 30 min. Cambogin was administered 14 times orally every 12 h from one day before the first challenge, meanwhile the control group mice were administered with PBS. Concentration of OVA-specific IgE in the serum was measured by ELISA. **(B)** In PSA test, mice were sensitized by i.v. injection of 2 μ g IgE in 100 μ l saline or treated with saline alone. After 24 h, the mice were challenged i.v with 2 mg DNP-HSA in 200 μ l saline after oral administration of 10 mg·kg⁻¹ cambogin for 1 h. Blood was collected 5 min after Ag challenge, and serum histamine concentration was determined by ELISA. All data are the means \pm s.e.m.. # $P < 0.05$ compared to non-treated mice. The experiments were performed twice with similar results and used a minimum of seven mice in each group.

Fig.S2

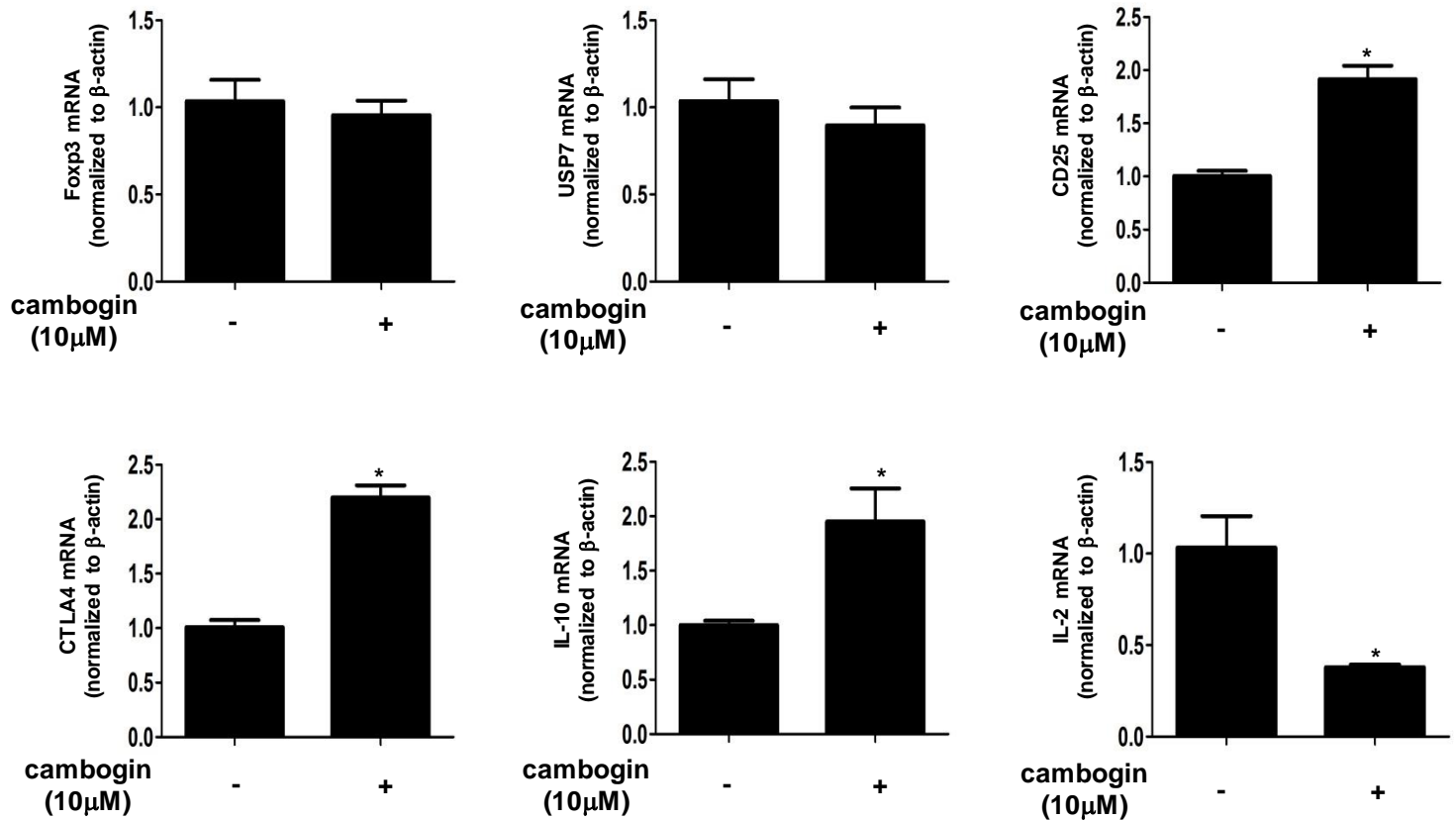


Fig.S2. Effect of cambogin on gene expression in primary human Treg cells. Human Treg cells were isolated from the PBMCs of healthy donors. The mRNA was prepared from these samples and used for the detection of Foxp3, USP7, CD25, CTLA4, IL-10 and IL-2 through qPCR. Data represent five independent experiments, and the error bars represent the means \pm SEM. Compared with untreated cells, * $P < 0.05$.

Fig.S3

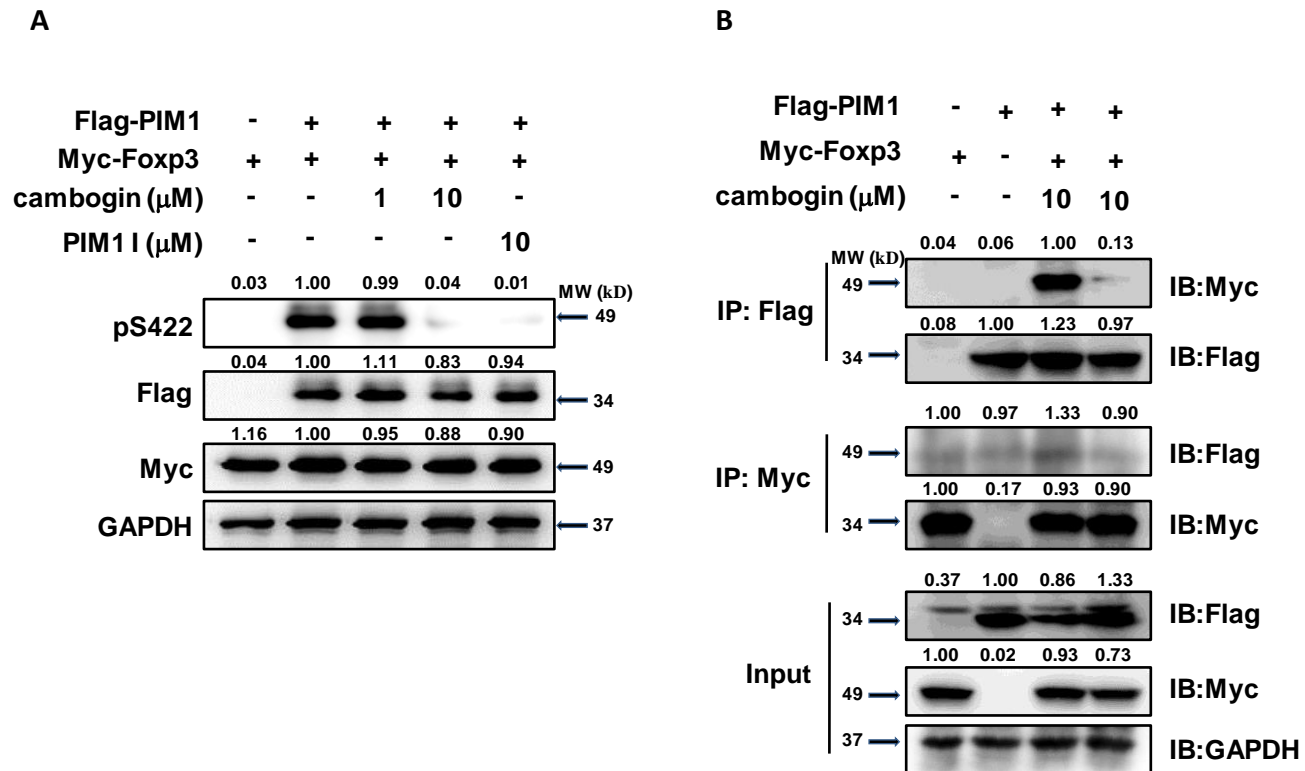


Fig.S3. HEK293 T cells were transfected with Myc-Foxp3 and Flag-PIM1, Cambogin was administered for 48 h after transfection. The indicated proteins were measured. The relative protein level were normalized to GAPDH by using Image J software. Data are representative of five independent experiments.

Fig.S4

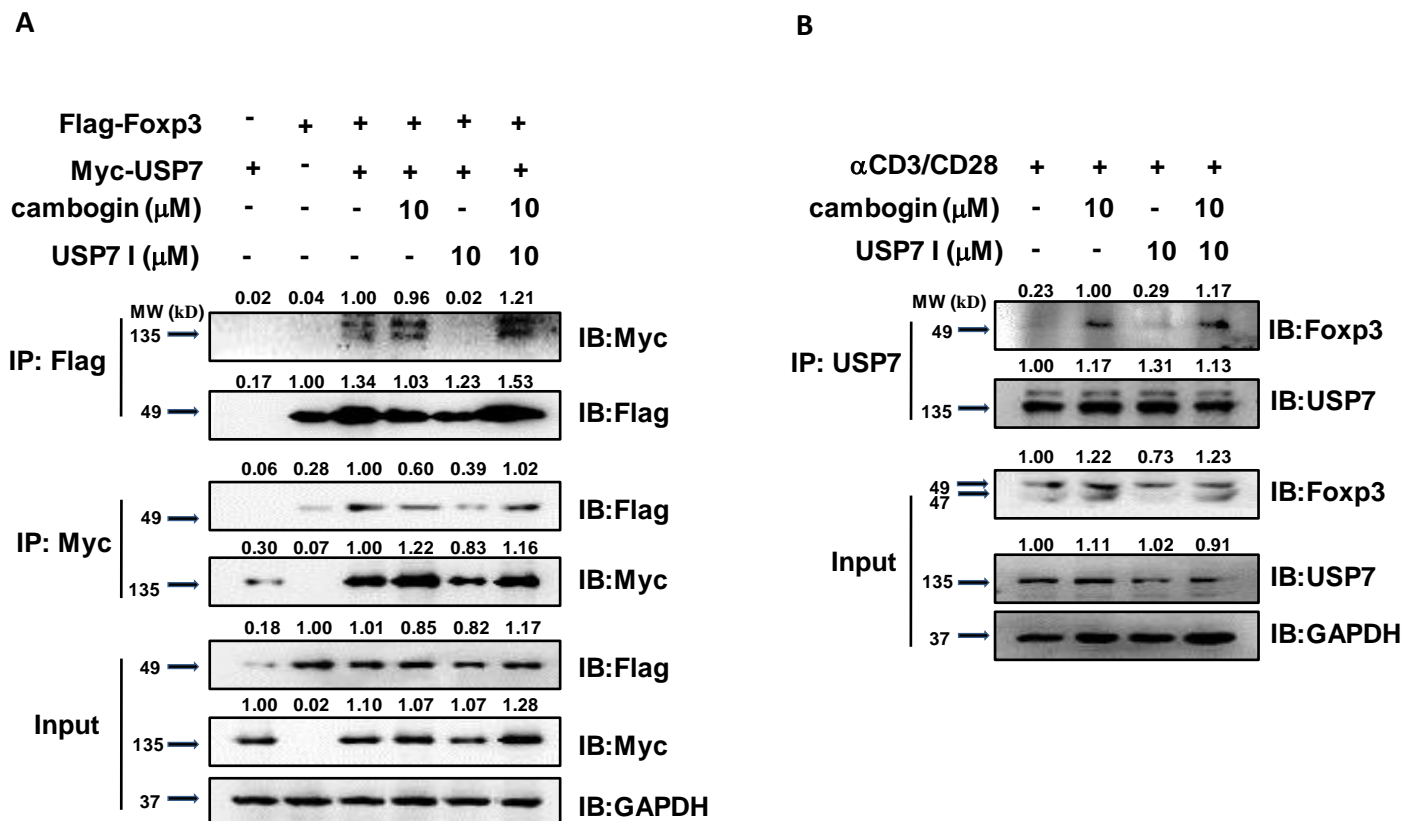


Fig.S4. Cambogin promoted the interaction between USP7 and Foxp3. (A) HEK293T cells were transfected with Flag-Foxp3 and Myc-USP7. Co-IP was performed using either anti-Flag antibody or anti-Myc antibody. (B) Primary human Treg cells were stimulated using anti-CD3 and anti-CD28 antibodies for 1 day after cambogin or USP7 inhibitor pretreatment. The cells were harvested and lysed using IP assay buffer. The cells lysate was immunoprecipitated with an anti-USP7 antibody. Immune blotting was performed with the indicated antibodies. The relative protein level were normalized to GAPDH by using Image J software. Data are representative of five independent experiments.

Fig.S5

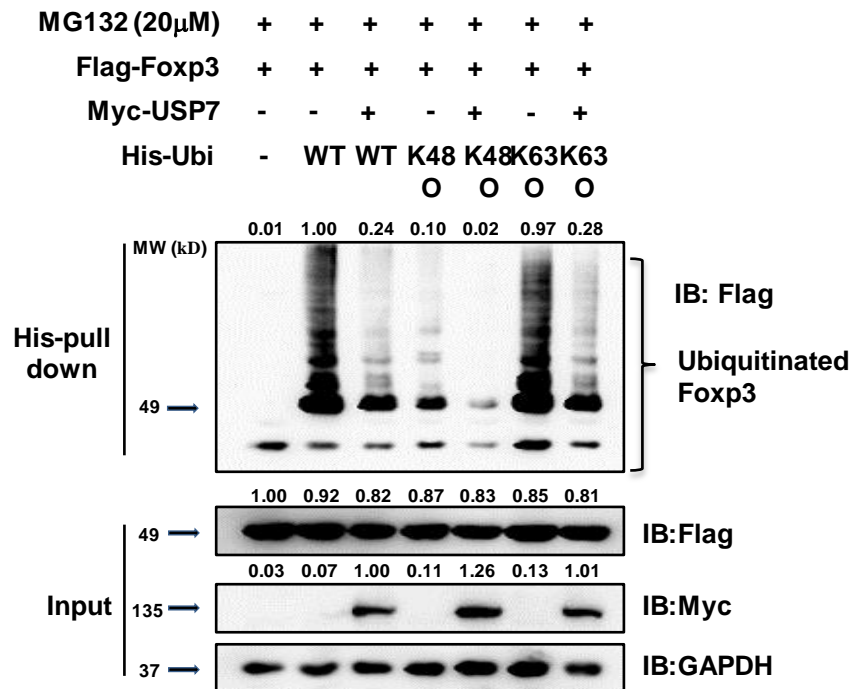


Fig.S5. HEK293 T cells were transfected with Myc-USP7, His-ubiquitin, Flag-Foxp3 or His-ubiquitin (WT, 48K, and 63K) and treated with 20 μ M MG132 for 4 h prior to harvest. Pull-down using Ni-NTA beads; ubiquitinated Foxp3 was visualized through IB using anti-Flag Ab. The relative protein level were normalized to GAPDH by using Image J software. Data are representative of five independent experiments.