### Interleukin-10
- **Forward**: 5′-CCCATCCTCGACGATCTC-3′
- **Reverse**: 5′-TCAGACTGGTTTGGGATAGGTTT-3′

### TGF-β1
- **Forward**: 5′-CTCCGTGCTTCTAGTGC-3′
- **Reverse**: 5′-GCCTTAGTTTGGACAGGATCTG-3′

### GAPDH
- **Forward**: 5′-CCAGGTTGTCTCTGCGACTT-3′
- **Reverse**: 5′-CCTGTTGCTGTAGCCGTATTCA-3′

### Foxp3-promoter
- **Forward**: 5′-CCCATTCCTCGTCACGATCTC-3′
- **Reverse**: 5′-TCAGACTGGTTTGGGATAGGTTT-3′

### Foxp3-CNS1
- **Forward**: 5′-CCAGGTTGTCTCTGCGACTT-3′
- **Reverse**: 5′-CCTGTTGCTGTAGCCGTATTCA-3′

### (For Taqman)

| Gene  | Cat#               |
|-------|-------------------|
| Hprt1 | Mm00446968_m1     |
| Foxp3 | Mm00475162_m1     |
| Tgf1  | Mm00441724_m1     |
| Il10  | Mm01288386_m1     |
| Tnf   | Mm00443258_m1     |
| Tgfb1 | Mm00436964_m1     |
| Tgfb2 | Mm03024091_m1     |
| Smad6 | Mm00484738_m1     |
| Smad7 | Mm00484742_m1     |
| Rel   | Mm01239661_m1     |
| Tbx1  | Mm00448949_m1     |
| Rorc  | Mm01261022_m1     |
| Nkfb1 | Mm00446968_m1     |
| Nkfb2 | Mm00476361_m1     |
| Ikbkb | Mm01222247_m1     |
| Jun   | Mm00495062_s1     |
| Il2ra | Mm01340213_m1     |
| Il2rb | Mm00434268_m1     |
| Smad2 | Mm00487530_m1     |
| Smad3 | Mm00489637_m1     |
| Smad4 | Mm03023996_m1     |
| Cd3e  | Mm01179194_m1     |
| Icos  | Mm00497600_m1     |
| Nkfbiz| Mm00600522_m1     |
| Cd44  | Mm01277161_m1     |
| Stat5a| Mm03053818_s1     |
| Stat5b| Mm00839899_m1     |
| Jak1  | Mm00600614_m1     |
| Hdac4 | Mm01299552_m1     |
| Ilng  | Mm01168134_m1     |
| Il2   | Mm00434256_m1     |
| Il5   | Mm00439646_m1     |
| Il13  | Mm00434204_m1     |
| Inha  | Mm00439683_m1     |
| Rorc  | Mm01261022_m1     |
| Batf  | Mm00479410_m1     |
| Stat3 | Mm00456961_m1     |
### Supplementary Table 1: Primers List

| Gene  | Primer ID       |
|-------|-----------------|
| Myd88 | Mm00440338_m1   |
| Myc   | Mm00487803_m1   |
| Hk1   | Mm00439344_m1   |
| Hk2   | Mm00443385_m1   |
| Socs1 | Mm00782550_s1   |
| Socs3 | Mm00545913_s1   |
| Slc2a1| Mm00441480_m1   |
| Slc2a3| Mm00441483_m1   |
| Runx1 | Mm01213404_m1   |
| Runx3 | Mm00490666_m1   |
| Tcf12 | Mm00441699_m1   |
| Cd274 | Mm03048248_m1   |
| Rara  | Mm01296312_m1   |
| Rora  | Mm01173766_m1   |
Supplementary Figure 1: Naive CD4\(^+\) T cells from C57/BL6 mice cultured in each concentration of GO-Y-030.

(A, B) Representative SSC and FSC FACS analysis at five independent experiments. Purified naïve CD4\(^+\) T cells cultured for three days, and then gated live cell population according to SSC and FSC. Statistical analyses were performed in each concentration between Curcumin and GO-Y030, and 2 ng/mL TGF-β versus all.
Supplementary Figure 2: Effects of GO-Y-030 in T cell viability.

(A) Representative Annexin V and Propidium Iodide FACS analyses at three independent experiments. Purified naïve CD4+ T cells cultured for three days, and then gated CD4+ cell population. (B, C) Statistic analyses were performed in each concentration between Curcumin and GO-Y030 versus DMSO control. One-way analysis of variance (ANOVA) with post-hoc Tukey’s multiple comparisons test employed. (D) Representative Annexin V and Propidium Iodide FACS
analyses at three independent experiments. Purified naïve CD8$^+$ T cells cultured for three days, and then gated CD8$^+$ cell population. (E, F) Statistic analyses were performed in each concentration between Curcumin and GO-Y030 versus DMSO control. One-way ANOVA with post-hoc Tukey’s multiple comparisons test employed.
Supplementary Figure 3: GO-Y030 prevent TGF-β induced Foxp3+Tregs generation

(A, B) Frequency of Foxp3+ Tregs in total CD4+ cells. Splenic naïve CD4+T cells were cultured in the presence or absence of 2 ng/mL TGF-β, 1 µM curcumin, or 0.1-0.025 µM GO-Y030 for three days. One-way analysis of variance (ANOVA) with post-hoc Tukey’s multiple comparisons test employed.
Supplementary Figure 4: Relative Foxp3^+ Tregs in CD4^+ Zombie Yellow^− T cells.

Splenic naïve CD4^+ T cells were cultured in the presence of 2 ng/mL TGF-β with or without curcumin or GO-Y030 for three days. Percentage of TGF-β-induced Foxp3^+ population is as set as “1”. One-way analysis of variance (ANOVA) with post-hoc Tukey’s multiple comparisons test employed.
Supplementary Figure 5: GO-Y030 prevents TGF-β-induced Foxp3⁺Tregs in human naïve CD4⁺ T cells.

(A) Human naïve CD4⁺ T cells were cultured in the presence of 2 ng/mL TGF-β1 with or without curcumin or GO-Y030 for three days. Data are one representative at three independent experiments. (B) Relative Foxp3 expression in CD4⁺ T cells. TGF-β1 stimulation only is set as “1”. One-way analysis of variance (ANOVA) with post-hoc Dunnet’s multiple (vs. TGF-β) comparisons test employed. (C) Relative live cell counts. Human naïve CD4⁺ T cells were cultured with or without 2 ng/mL TGFβ and concentrations of Curcumin or GO-Y030 as indicated for 72 h followed by the addition of the cell counting reagent. Red: No cells (Medium alone). Data are representative at three independent experiments using different healthy donors. One-way ANOVA with post-hoc Dunnet’s multiple (vs. TGF-β) comparisons test employed.
**Supplemental Figure 6: GO-Y030 does not affect TGF-β-induced SMAD pathway.**

(A) Representative western blotting image of SMAD3, phospho-SMAD3 and GAPDH at three independent experiments. Naive CD4\(^+\) T cells were stimulated with or without TGF-β in the presence or absence of 0.1 uM GO-Y030 or 1 uM Curcumin. (B) Relative SMAD3 and phospho-SMAD3 expression. (n=3, Mean with standard error of the mean) Without TGF-β and GO-Y030 stimulation was set as “1”. One-way analysis of variance (ANOVA) with post-hoc Tukey’s multiple comparisons test employed. One-way ANOVA with post-hoc Tukey’s multiple comparisons test employed.
Supplemental Figure 7: Relative murine Foxp3-promoter activity

(A) pGL4-Foxp3 promoter (-1702 to +174) activity were analyzed using the Duo-luciferase assay systems. DMSO- or 1.0 μM GO-Y030 were treated HEK293 before 24 h electroporation. pcDNA3-empty vector transfection (Firefly/Renilla) was set as “1”. (B) pGL4-basic promoter (control) activity was analyzed using the Duoluciferase assay systems. DMSO- or 1.0 μM GO-Y030 were treated HEK293 cells before 24 h transfection. Data are one representative at two independent experiments (n=2, Mean + standard deviation).
Supplemental Figure 8: Representative Foxp3 expression in Tregs after CD4⁺CD25⁺ isolation.

(A, B) Splenic CD4⁺CD25⁺ Tregs were isolated by using autoMACS (Milteny Biotech) and FACS analyses. Data are one representative of three independent experiments.
Supplemental Figure 9: Phenotype of Cultured CD4⁺CD25⁺ Tregs.

(A) Foxp3 Mean fluorescence Intensity in CD4⁺Foxp3⁺ Treg populations in Figure 3A. Data shows six independent experiments. (B, C) Relative cell survival rate after 18 h culture of CD4⁺CD25⁺ Tregs with or without Curcumin or GO-Y030. Data are one representative at more than three independent experiments. (D) Absolute number of cells after 18 h culture of CD4⁺CD25⁺ Tregs with or without Curcumin or GO-Y030. The starting number of cells in each wells was 1 x 10⁵ cells. (E) Foxp3-GFP positive cells in CD4⁺ population. Foxp3-GFP positive cells were purified by FACS-Aria II (>95%) and cultured 18 h with CD3 + CD28. Data are one representative at three independent experiments. GITR (F) and CTLA4 (G) in CD4⁺Foxp3⁺ Treg populations in Figure 5A. Gray; Isotype control, Black; GITR or CTLA4. Data are one representative at three independent experiments. One-way analysis of variance with Tukey employed for statistic difference.
Supplemental Figure 10: GO-Y030 controls IL-2/STAT5 axis in CD4+CD25+ Tregs.

(A) Percentage of Foxp3 in cultured CD4+CD25+ Tregs at day three. Data are representative at five independent experiments. Student T-test was employed. (B) Percentage of Foxp3 in cultured CD4+CD25+ Tregs in the suppression assay. DMSO- or GO-Y030-treated CD4+CD25+ Tregs were co-cultured with CD8+ T cells (Tregs:CD8+ T cells=0.5:1) for three days. Data are representative at shows five independent experiments. Student T-test was employed.
Supplemental Figure 11: GO-Y030 controls IL-2/STAT5 axis in CD4^+CD25^+ Tregs.

(A) Real time PCRs in 72 h culture of CD4^+CD25^+ Tregs with or without Curcumin or GO-Y030. The color scale is shown at the top of heat map. Each gene expression of DMSO-treated Tregs are as set as “1”. Data showed four independent experiments. (B, C) Enrichr- was used to calculate enrichment scores of signaling pathways. We selected genes of significantly difference expression (P<0.05) between DMSO-Tregs and GO-Y030-Tregs (B, https://maayanlab.cloud/Enrichr/enrich?dataset=ce1ae4783a07360aa829b0fd36eb1) or Curcumin-Tregs and GO-Y030-Tregs (C, https://maayanlab.cloud/Enrichr/enrich?dataset=35a8b176b017fbdc7c09576aa75995cd). Statistical analyses (One-way analysis of variance with Tukey) were performed.
Supplemental Figure 12: GO-Y030 controls IL-2/STAT5 axis in CD4^+Foxp3-GFP^+ Tregs.

(A) Real time PCRs in 72 h culture of CD4^+Foxp3-GFP^+ Tregs with or without 1 μm Curcumin or 0.25 μm GO-Y030. Data pooled three independent experiments. Statistical analyses (One-way analysis of variance with Tukey) were performed. (B). Th17 population in cultured CD4^+Foxp3-GFP^+ Tregs with or without 0.25 μm GO-Y030. Student T-test was performed.
Supplemental Figure 13: GO-Y030 did not prevent infiltration and activation of CD8⁺ cells in tumor microenvironment.

(A) Frequency of CD4⁺/CD8α⁺ cells in tumor infiltrate lymphocytes. (B) Ratio of tumor infiltrate CD8⁺ cells to CD4⁺Foxp3⁺ Treg cells. (C, D) Ki67 expression in CD8α⁺ cells in tumor infiltrate lymphocytes. Red; isotype control. Data are one representative of each of the two independent experiments (A, C). (E, F) IFN-γ production from CD8α⁺ cells in tumor infiltrate lymphocytes. Red; isotype control. (G, H) TNF-α production from CD8α⁺ cells in tumor infiltrate lymphocytes. Red; isotype control. One-way analysis of variance with post-hoc Tukey’s multiple comparisons test was used (B, D, F, H). The graph shows mean and standard deviation.