Supplemental data

Redirecting TGF-β signaling via β-catenin/Foxo prevents kidney fibrosis

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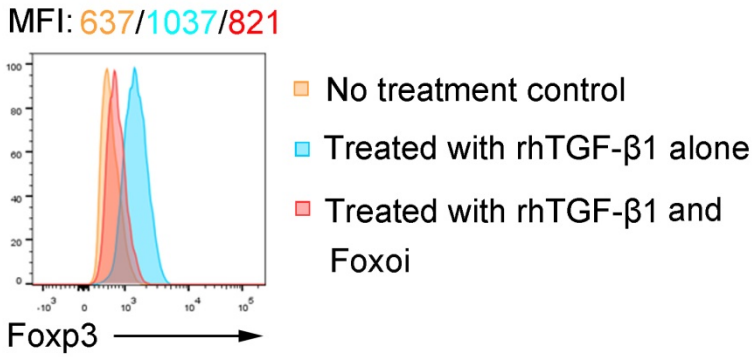
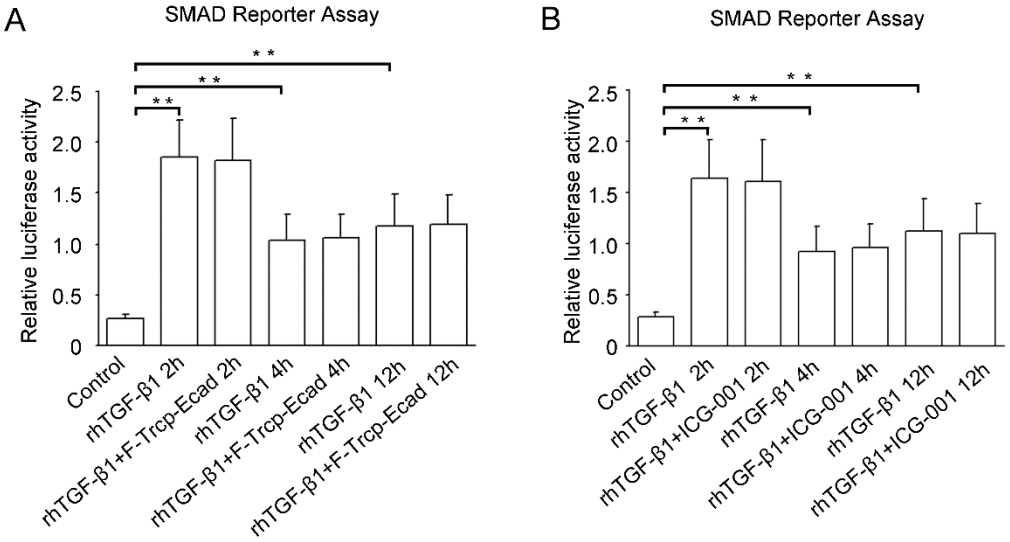
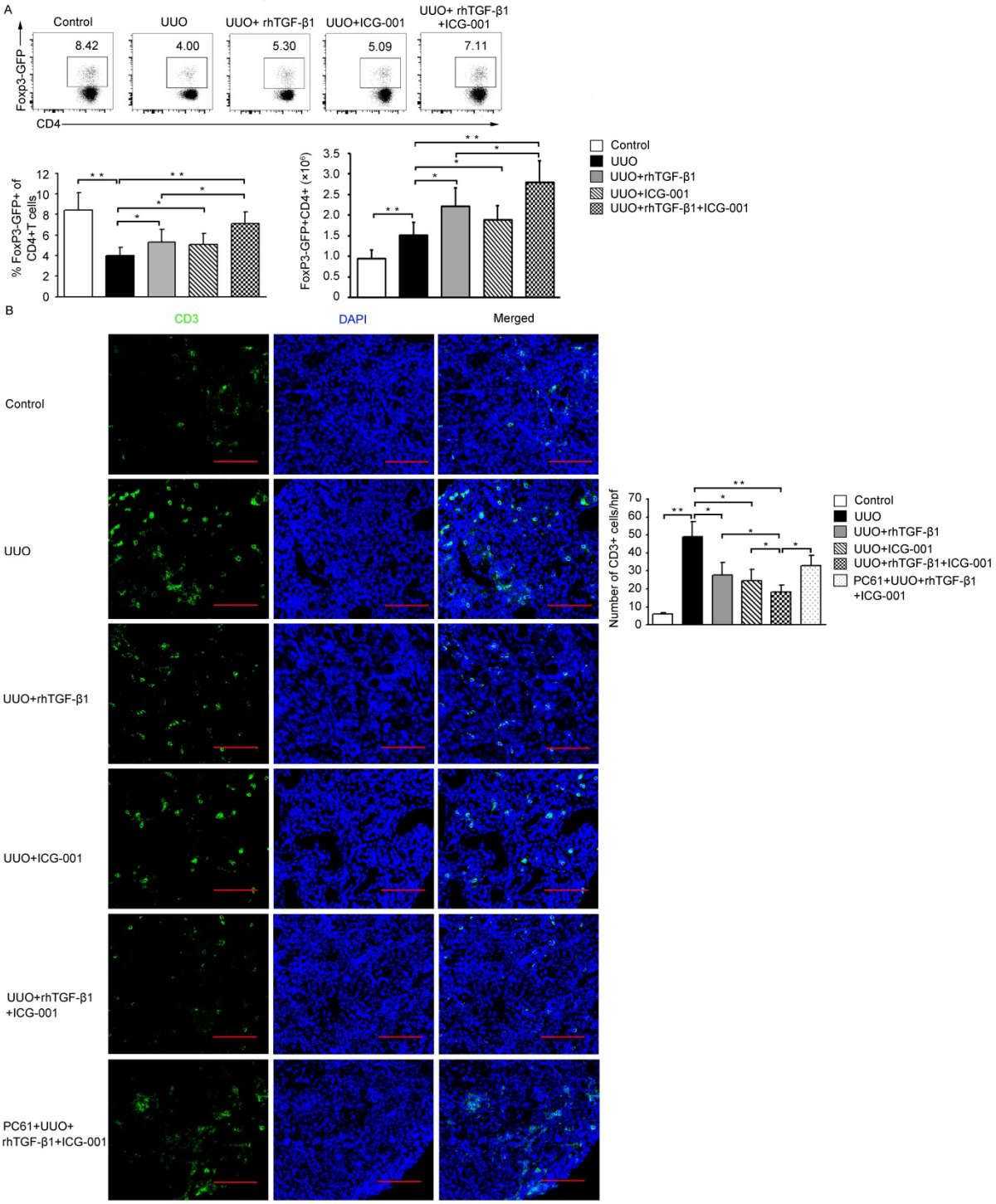
Supplemental Figures



Supplemental Figure 1. Representative histogram analyses of Foxp3 in iTregs arising from EL4 cells treated with rhTGF-β1 with and without Foxo inhibitor (Foxoi) AS1842856 (25 nM). MFI: mean fluorescence intensity.

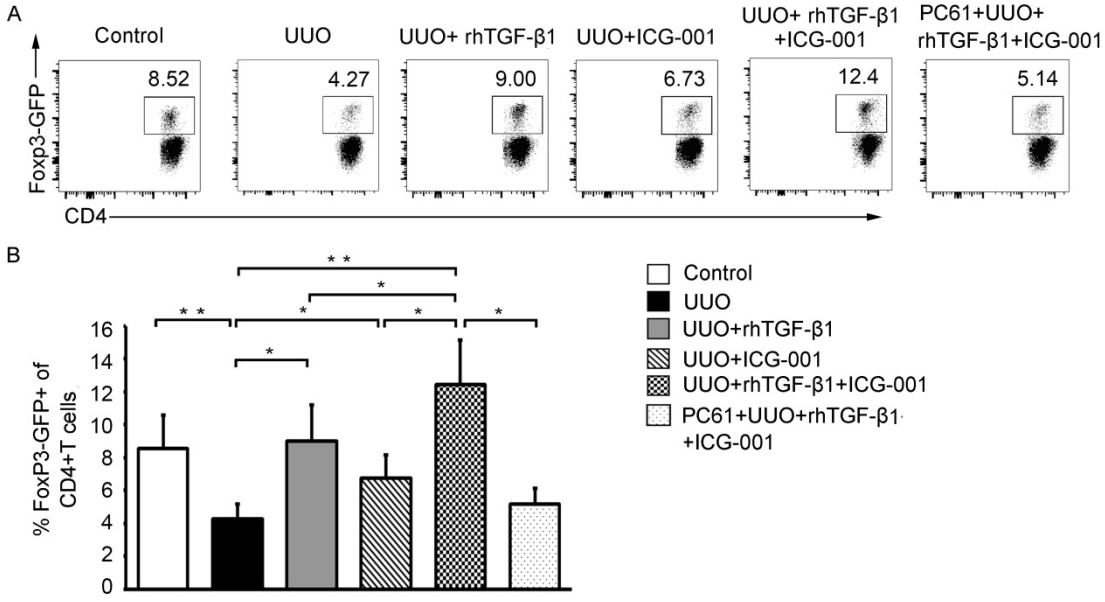


Supplemental Figure 2. SMAD reporter activity in rhTGF-β1-treated EL4 cells is not changed by β-catenin. (A) The SMAD reporter (relative luciferase activity) assay in control EL4 cells, and EL4 cells treated with rhTGF-β1 or rhTGF-β1 with F-TrCP-Ecad-transfection. **(B)** The SMAD reporter assay in EL4 cells in control EL4 cells, and EL4 cells treated with rhTGF-β or rhTGF-β1 and ICG-001. Bars represent mean ± SEM of triplicate determinations. Statistical significance was determined by one way ANOVA, followed by Tukey's post-hoc test. ** $P < 0.01$.

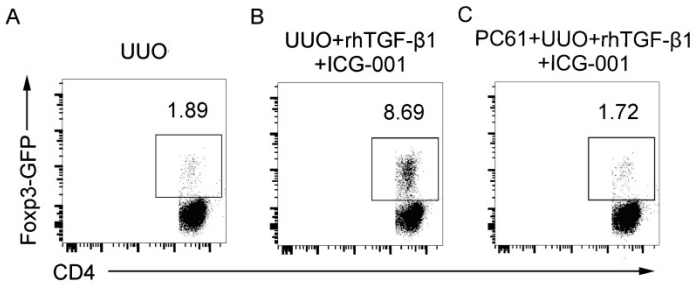


Supplemental Figure 43. Shift of β-catenin from TCF to Foxo reduces kidney CD3$^+$ T cell infiltration in UUO mice by TGF-β-induced Tregs. (A) Representative flow cytometry plots (gating on CD3$^+$ CD4$^+$ cells) and bar graph showing the percentage and the absolute number of Tregs in the spleen at day 3 after UUO (one of five experiments is shown). A pool of splenocytes from 1 spleen was analyzed in each group. **(B)** Representative immunofluorescence staining and quantitation of CD3+ T cell infiltration in kidneys. Original magnification, × 40 (n=6/group). Statistical significance was determined by one way

ANOVA, followed by Tukey's post-hoc test. All data are expressed as the mean ± SEM. * P ＜0.05, **P＜0.01. Scale bars: 100 μm.



Supplemental Figure 54. Shift of β-catenin from TCF to Foxo increases Tregs in spleen of UUO mice. Representative flow cytometry plots (gating on $CD3^+$ $CD4^+$ cells) (A) and bar graphs (B) showing the percentage of Tregs in spleen at day 7 after UUO (one of five experiments is shown). A pool of splenocytes from 1 spleen was analyzed in each group. Statistical significance was determined by one way ANOVA, followed by Tukey's post-hoc test. All data are expressed as the mean ± SEM. * P＜0.05, **P＜0.01.



Supplemental Figure ~~3~~5. Representative flow cytometry plots (gating on $CD3^+$ $CD4^+$ cells) of $CD4^+$Foxp3-GFP^+ Tregs in the spleen of untreated **(A),** control isotype IgG-injected **(B)** and PC61 injected **(C)** UUO mice treated with or without rhTGF-β1 and ICG-001.