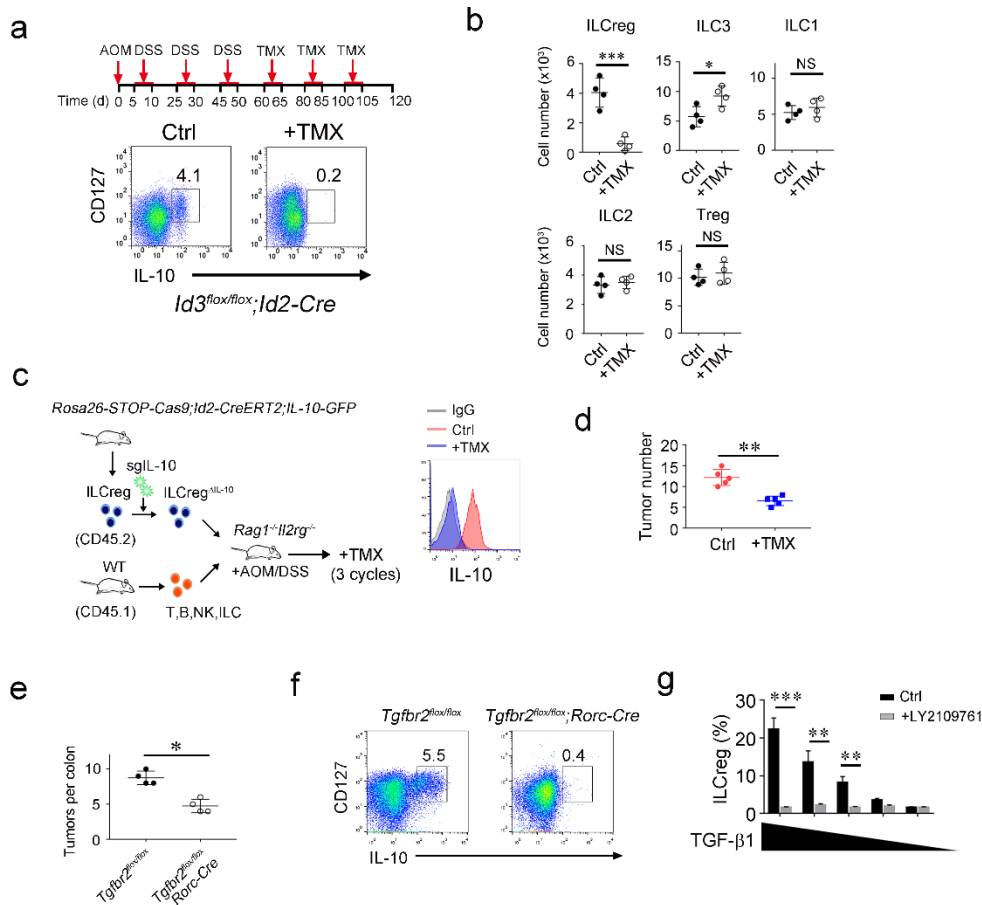


Supplementary information Fig. S5



Supplementary information Fig. S5. TGF- β signaling is required for the conversion of ILC3 to ILCreg. (a) $Id3^{flox/flox}; CreERT2$ mice were treated as described. At day 120, colon tumor cells were isolated and ILCreg (Lin-CD45⁺CD127⁺IL-10⁺) cells were analyzed by flow cytometry. (b) Analysis of indicated lymphocytes from $Id3^{flox/flox}; CreERT2$ mice with indicated treatment by flow cytometry. Cell numbers were calculated and shown as means \pm SD. *, $P < 0.05$; **, $P < 0.01$; NS, not significant by Two-tailed unpaired Student's t -test. $n = 5$ for each group. (c) Adoptive transfer assays with IL-10-deleted ILCregs (ILCreg $^{\Delta IL-10}$). ILCregs from $Rosa26-STOP-Cas9; Id2-ERT2-Cre; IL-10-GFP$ mice were isolated and infected with lentivirus carrying sgRNA against *Il10* gene. Infected cells were selected with 1 μ g/ml puromycin and transferred into $Rag1^{-/-}; Il2rg^{-/-}$ mice together with respective WT (CD45.1) lymphocytes. IL-10 expression in ILCregs (CD45.2) after treatment with TMX was detected prior to transplantation (right panel) by flow cytometry. ILCregs infected with lentivirus carrying empty vector were used as a negative control (Ctrl). (d) ILCreg $^{\Delta IL-10}$ transferred mice were subjected to AOM/DSS treatment. At day 60, mice were treated with TMX for 3 cycles as in (a). At day 120, tumors in colon from indicated mice were calculated and shown as means \pm SD. **, $P < 0.01$ by Two-tailed unpaired Student's t -test. $n = 5$ for each group. (e-f) $Tgfb2^{flox/flox}$ and $Tgfb2^{flox/flox}; Rorc-Cre$ mice were subjected to AOM/DSS treatment. Tumor numbers were calculated and shown

as means \pm SD. **, $P < 0.01$. Two-tailed unpaired Student's t -test was used. $n=5$ for each group (e). ILCreg (Lin⁻CD45⁺CD127⁺IL-10⁺) from colon tumors of indicated mice were analyzed by flow cytometry (f). (g) TdTomato⁺ILC cells (tdTomato⁺CD127⁺CD45⁺Lin⁻) were isolated from AOM/DSS induced colon tumors from *Rosa26-STOP-tdTomato;Rorc-Cre;IL-10-GFP* mice. TdTomato⁺ILC cells treated with different doses of TGF- β (from 4 ng/ml to 20 ng/ml) in complete cell culture in the presence or absence with 2 μ M TGF- β inhibitor LY2109761. After 7 days, cultured cells were analyzed by flow cytometry for IL-10-GFP⁺ ILCreg cells. Percentage of ILCreg cells were calculated and shown as means \pm SD. **, $P < 0.01$ Two-tailed unpaired Student's t -test. Data are representative of at least four independent experiments.