

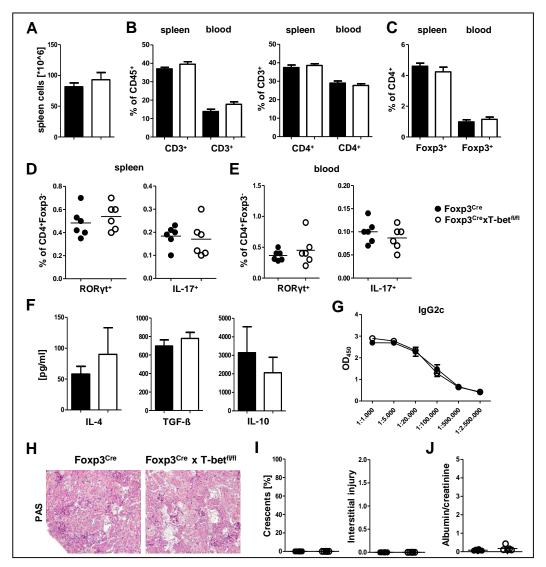
SUPPLEMENTARY FIGURE S1. Dynamics of renal and splenic Tbet⁻ Tregs.

(A, B) Quantification of renal T-bet⁻Foxp3⁺ Tregs as percentages of (A) CD4⁺ or

(B) Foxp3⁺ cells at the indicated time-points after induction of NTN. (C+D)

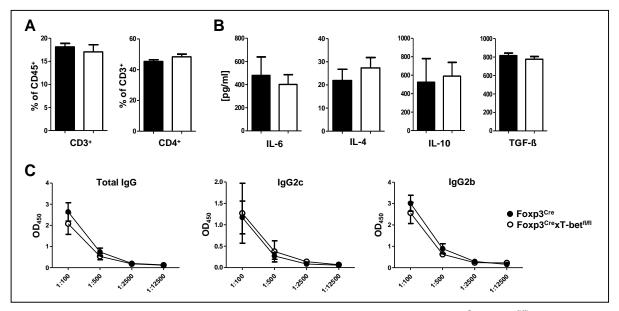
Quantification of splenic T-bet⁻Foxp3⁺ Tregs as percentages of (C) CD4⁺ or (D)

Foxp3⁺ cells at the indicated time-points after induction of NTN. n≥5 mice were analyzed per time-point.



SUPPLEMENTARY FIGURE S2. Characterization of naïve Foxp3^{Cre}xT-betfl/fl mice

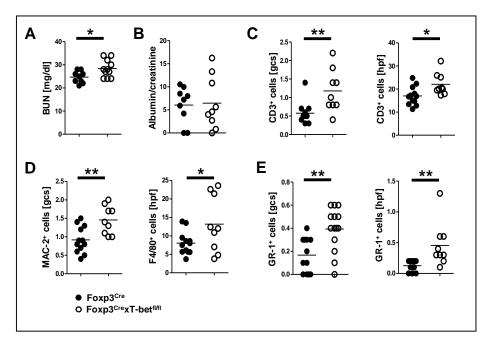
Analyses derive from 11-13 week old naïve Foxp3^{Cre} and Foxp3^{Cre}xT-bet^{fl/fl} mice. (A) Quantification of total spleen cells. (B) FACS analysis of spleen and blood CD3⁺ and CD4⁺ T cell populations. (C) FACS analysis of spleen and blood Foxp3⁺ Treg percentages. (D, E) Quantification of Th17 cells expressing RORyt or IL-17 from (D) spleens or (E) blood. (F) Spleen cell production of the indicated cytokines as measured by ELISA. (G) Serum levels of total IgG2c shown as OD at 450nm in serial dilutions as indicated. (H) Representative photographs of PAS-stained kidney sections (original magnification x200). (I) Quantification of glomerular crescents and interstitial injury. (J) Urinary albumin/creatinine ratio. n=6 vs 6 mice were analyzed in (A-J).



SUPPLEMENTARY FIGURE S3. Systemic immunity in antigen challenged Foxp3^{Cre}xT-bet^{fl/fl} mice

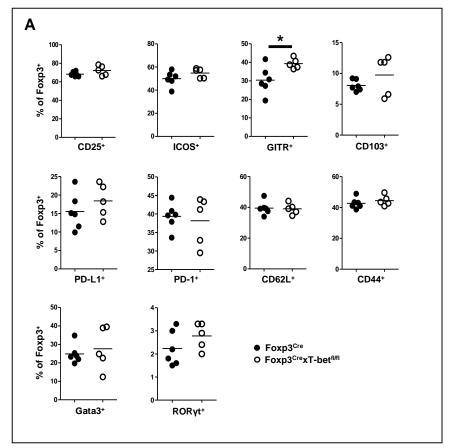
(A) FACS analysis of spleen CD3⁺ and CD4⁺ T cell populations. (B) Spleen cell production of the indicated cytokines as measured by ELISA. Analyses in (A, B) were performed at day 15 after NTN induction. 9

Foxp3^{Cre} vs 11 Foxp3^{Cre}xT-bet^{fl/fl} mice were analyzed. (C) Serum levels of anti-sheep globulin specific total IgG, IgG2c and IgG2b at day 12 after sheep IgG immunization. Data are shown as OD at 450nm in serial dilutions as indicated. 5 Foxp3^{Cre}vs 5 Foxp3^{Cre}xT-bet^{fl/fl} mice were analyzed.



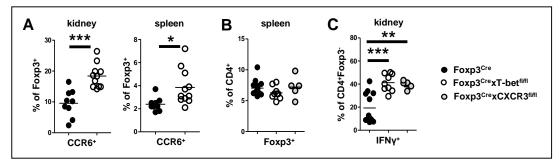
SUPPLEMENTARY FIGURE S4. Aggravation of NTN in the absence of Treg1 cells

(A) Quantification of blood urea nitrogen (BUN) at day 15 after NTN. (B) Urinary albumin/creatinine ratio at day 15 after NTN. (C) Glomerular and interstitial CD3+ T cells at day 22 after NTN. (D) Glomerular MAC-2+ and interstitial F4/80+ Macrophages at day 22 after NTN. (E) Glomerular and interstitial GR-1+ neutrophils at day 22 after NTN. In (A) 9 Foxp3^{Cre} vs 11 Foxp3^{Cre}xT-bet^{fl/fl} mice, in (B) 9 Foxp3^{Cre} vs 9 Foxp3^{Cre}xT-bet^{fl/fl}, in (C-E) 12 Foxp3^{Cre} vs 9 Foxp3^{Cre}xT-bet^{fl/fl} mice were analyzed. *P<0.05, **P<0.01.



SUPPLEMENTARY FIGURE S5.

(A) FACS analysis of the indicated surface molecules and transcription factors from splenic Tregs of Foxp3^{Cre}xT-bet^{fl/fl} or Foxp3^{Cre} control mice at day 12 after immunization with sheep IgG. 6 Foxp3^{Cre} vs 5 Foxp3^{Cre}xT-bet^{fl/fl} mice were analyzed. *P<0.05.



SUPPLEMENTARY FIGURE S6.

(A) FACS analysis of CCR6 expression on renal and splenic Tregs in nephritic animals at day 15 of NTN. 9 Foxp3^{Cre} vs 11 Foxp3^{Cre}xT-bet^{fl/fl} mice were analyzed. (B) FACS analysis of Treg percentages in spleens of the indicated mouse lines at day 22 of NTN. (C) FACS analysis of IFNy producing Th1 cells in kidneys of the indicated mouse lines at day 22 of NTN. In (B+C), 12 Foxp3^{Cre} vs 9 Foxp3^{Cre}xT-bet^{fl/fl} vs 5 Foxp3^{Cre}xCXCR3^{fl/fl} mice were analyzed. *P<0.05, **P<0.01, ***P<0.001.