Supplemental Data

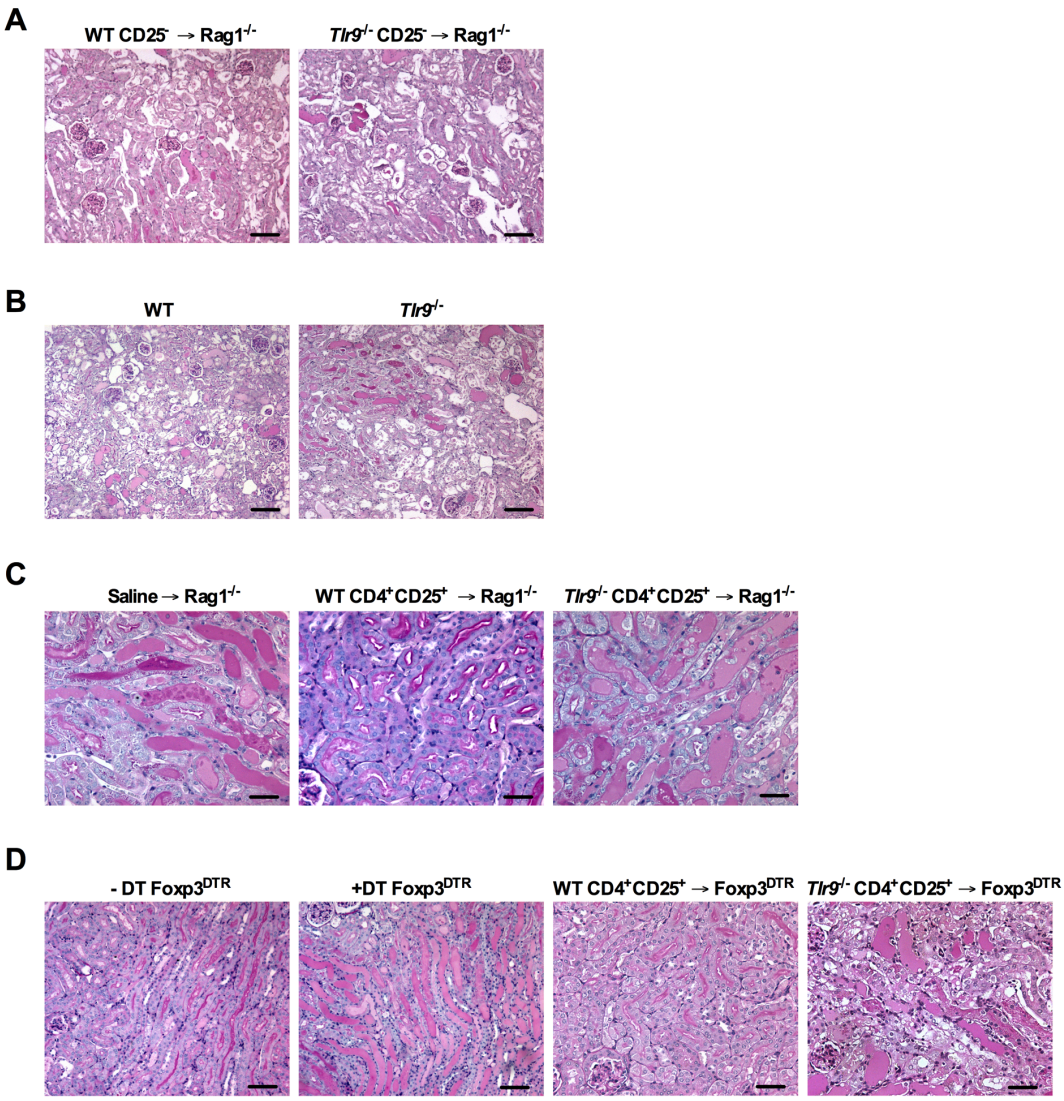
Supplemental Table 1. Gene and protein expression of chemokine receptors on $CD4^+CD25^+$ cells from naïve WT and *Tlr9*$^{-/-}$ mice.

	Gene expression WT vs. *Tlr9*$^{-/-}$ $CD4^+CD25^+$ cells (mRNA, RQ values)	Cell surface expression WT vs. *Tlr9*$^{-/-}$ (Flow cytometry, % of $CD4^+CD25^+$ cells
CCR4	1.02±0.12 vs. 1.51±0.36, NS	10.1±1.17 vs. 9.51±0.93[a], NS
CCR6	1.00±0.05 vs. 1.38±0.07**	74.7±3.24 vs. 66.7±5.28[a], NS
CCR7	1.00±0.04 vs. 2.38±0.63, NS	98.9±0.18 vs. 99.1±0.18[b], NS
CXCR3	1.02±0.10 vs. 2.37±0.66, NS	34.9±1.92 vs. 37.9±1.12[b], NS

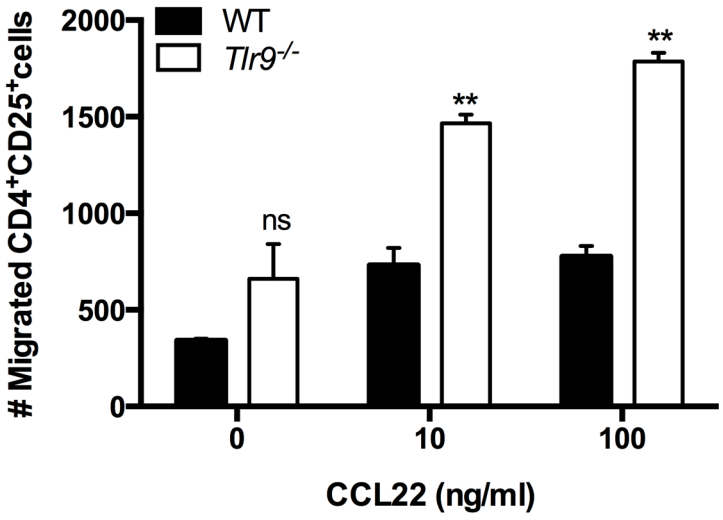
The left column shows mRNA gene expression data of chemokine receptors important in Treg recruitment to sites of inflammation on sorted $CD4^+CD25^+$ cells from naïve WT and *Tlr9*$^{-/-}$ spleens. *n*=4 in each group. Gene of interest is relative to 18S and presented as relative quantification (RQ) to WT $CD4^+CD25^+$ cells. The right column shows flow cytometry data of cell surface expression of chemokine receptors on $CD4^+CD25^+$ cells from the spleen or blood in naïve WT and *Tlr9*$^{-/-}$ mice. *n*=5 in each group. Values represent mean ± SEM (unpaired Student's *t* test). WT, wild-type, NS, non significant. **, $P < 0.01$.

[a] $CD4^+CD25^+$ cells in the blood were investigated

[b] $CD4^+CD25^+$ cells in the spleen were investigated



Supplemental Figure S1. Representative photomicrographs of PAS-stained kidney sections from reconsitution and depletion studies after cisplatin adminsitration in Figure 2. (A) Kidney sections after cisplatin administration showed equivalent tubular damage with tubular cast formation and matrix expansion in both reconstituted groups. Scale bar, 100 μm. (B) Kidney sections showed a severe but equivalent pattern and intensity of renal injury in $CD25^+$ cell-depleted WT and *Tlr9*$^{-/-}$ mice Scale bar, 100 μm. (C) Representative photomicrographs demonstrate more tubular injury in *Rag1*$^{-/-}$ mice reconstituted with *Tlr9*$^{-/-}$ $CD4^+CD25^+$ regulatory cells and saline injection after cisplatin administration. Scale bar, 50 μm. (D) Kidney sections showing greater renal tubular damage in $Foxp3^{DTR}$ mice reconstituted with *Tlr9*$^{-/-}$ $CD4^+CD25^+$ cells and control mice receiving diphtheria toxin (DT) injections compared to mice reconstituted WT $CD4^+CD25^+$ cells and control mice not treated with DT. Scale bar, 100 μm.



Supplemental Figure S2. *Tlr9*$^{-/-}$ CD4^{+}CD25^{+} T cells do not have reduced migration toward the inflammatory chemokine CCL22. The chemotactic response of WT and *Tlr9*$^{-/-}$ CD4^{+}CD25^{+} T cells to increasing concentration of CCL22 was examined in an *in vitro* chemotaxis assay. The number of migrating cells was measured by flow cytometry acquisition of a fixed number of beads. Data represents mean ± SEM (Two-way ANOVA). NS, not significant; **, $P < 0.01$.