Supplemental Information

Tolerogenic dendritic cells attenuate experimental

autoimmune anti-myeloperoxidase glomerulonephritis

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Supplemental Table 1. The effect of DMSO-treated MPO-pulsed DCs on anti-MPO immunity and glomerulonephritis

	Vehicle (saline; n = 6)	DMSO[a]-treated MPO-pulsed DCs[b] (n = 6)	P value
% abnormal glomeruli	48.8 ± 4.4	46.3 ± 7.1	0.76
Proteinuria (µg/24h)	1425 ± 105	1539 ± 276	0.69
Skin DTH[c] (Δmm)	0.2 ± 0.02	0.2 ± 0.03	0.46
CD4 T cells			
% Ki-67+ cells	9.5 ± 1.8	7.3 ± 1.0	0.30
% AnV[d]+PI[e]- cells	10.5 ± 1.4	7.1 ± 0.7	0.049
% IL-17A+ cells	1.2 ± 0.5	0.8 ± 0.2	0.41
IL-17A MFI[f]	195.3 ± 13.1	193.8 ± 20.3	0.95
% IFNγ+ cells	2.3 ± 0.2	2.7 ± 0.3	0.26
IFNγ MFI	53.0 ± 6.8	41.7 ± 3.8	0.18
% IL-4+ cells	0.2 ± 0.03	0.2 ± 0.02	0.34
IL-4 MFI	158 ± 22.1	187.7 ± 30.3	0.45
CD8 T cells			
% Ki-67+ cells	6.3 ± 1.4	7.5 ± 2.0	0.62
% AnV+PI- cells	2.6 ± 0.3	1.9 ± 0.03	0.048
% IL-17A+ cells	0.3 ± 0.1	0.5 ± 0.1	0.25
IL-17A MFI	42.5 ± 3.0	47.2 ± 4.9	0.43
% IFNγ+ cells	3.5 ± 0.3	5.0 ± 0.7	0.08
IFNγ MFI	251.2 ± 27.0	194.2 ± 25.0	0.15
% IL-4+ cells	0.12 ± 0.02	0.29 ± 0.05	0.008
IL-4 MFI	45.7 ± 5.5	38.2 ± 4.2	0.31
B cells			
% Ki-67+ cells	13.2 ± 1.1	16.1 ± 1.3	0.11

% AnV+PI- cells	13.5 ± 1.6	10.6 ± 0.7	0.13

DMSO-treated MPO-pulsed DCs or vehicle (saline) were administered to mice with established anti-MPO immunity (day 14), 7 days before triggering GN. Anti-MPO immunity (in lymph nodes) and disease were assessed on day 26.

[a] DMSO, dimethylsulfoxide.
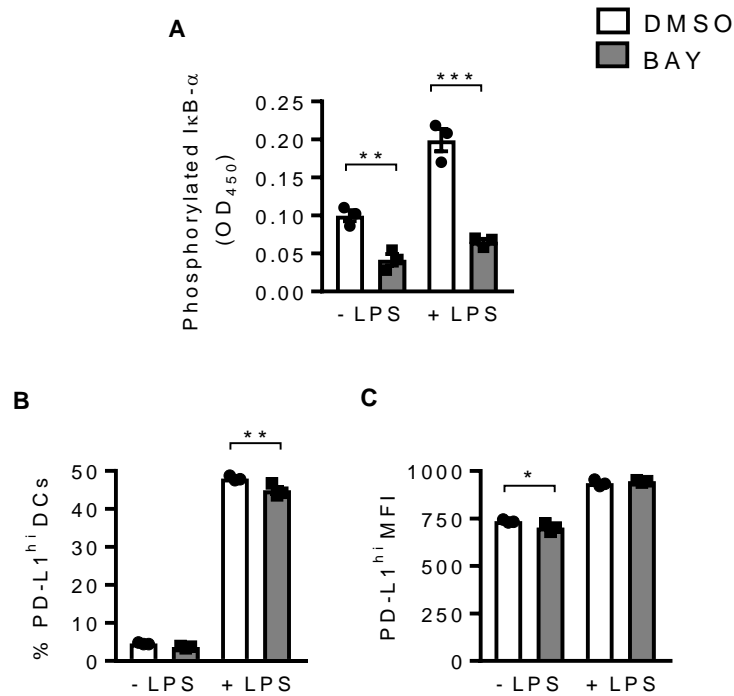
[b] DCs, dendritic cells.

[c] DTH, delayed type hypersensitivity.

[d] AnV, Annexin V.

[e] PI, propidium iodide.

[f] MFI, mean fluorescence intensity.

Results are presented as the mean ± SEM.

Supplemental Figure 1. The effect of BAY on DC phospho-IκBα levels and expression of PD-L1.

DMSO or BAY-treated DCs were cultured with or without LPS for 24 hours. Phosphorylated IκBα

levels where measured by ELISA using DC lysates, while DC expression of PD-L1 was assessed by

flow cytometry. (A) Levels of phosphorylated IκBα. (B) The proportion of DCs expressing PD-L1.

(C) The level of DC expression of PD-L1. DC, dendritic cells. LPS, lipopolysaccharide. DMSO,

dimethylsulfoxide. MFI, mean fluorescence intensity. Data are presented as scatter plots with the mean

± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
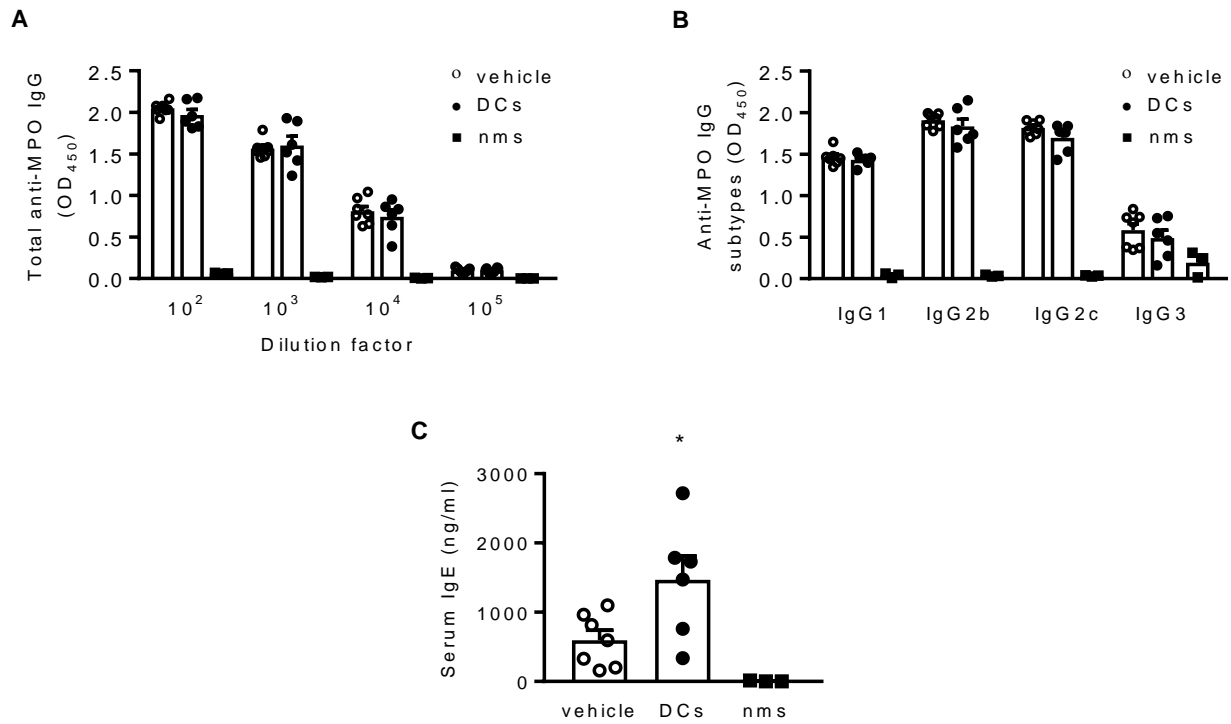
Supplemental Figure 2. The effect of tolerogenic DCs on antibody levels. Mice were immunised with MPO/adjuvant (days 0 and 7) and GN triggered on day 21. BAY-treated MPO-exposed DCs (MPO/BAY DCs; n = 6) or vehicle (saline; n = 7) were administered on day 14 and serum anti-MPO antibody titres and total IgE levels measured by ELISA on day 26. (A) Total anti-MPO IgG. (B) Anti-MPO IgG subtypes (at 1/100 dilution). (C) Total IgE. Data are presented as scatter plots with the mean ± SEM. nms, normal mouse serum. * *P* < 0.05.
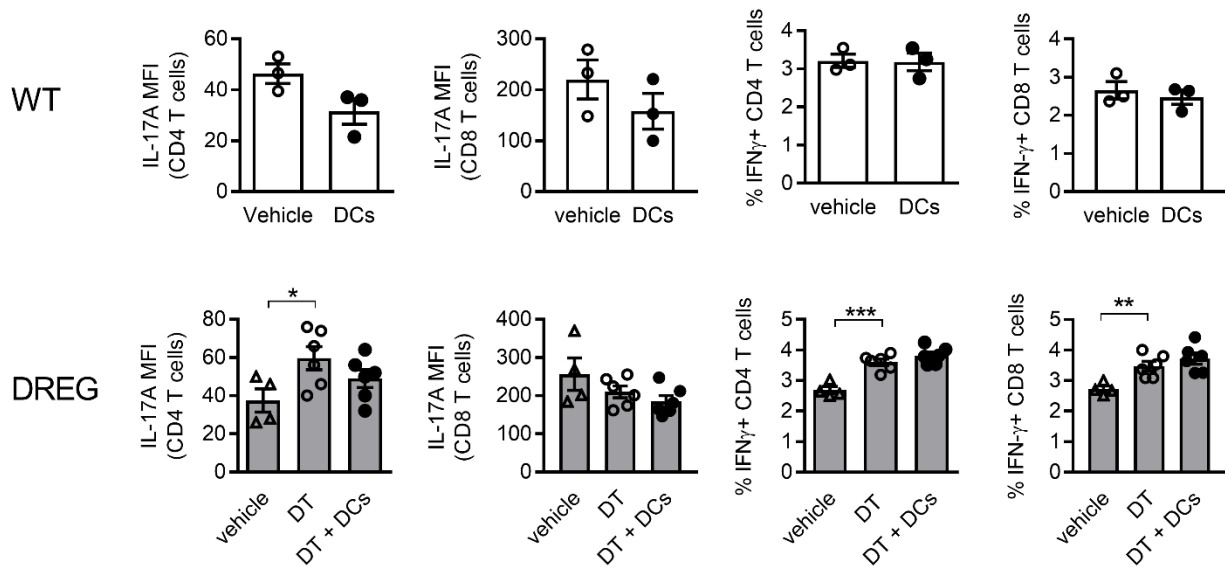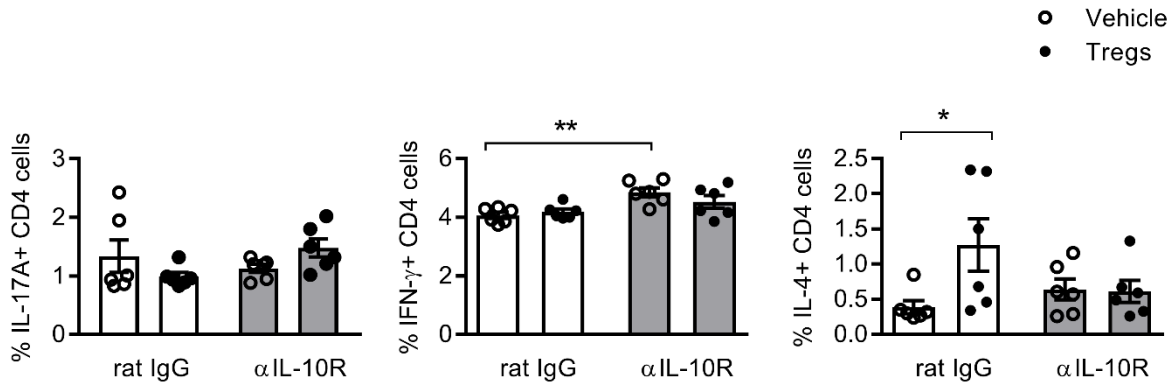
Supplemental Figure 3. T cell cytokine production in wildtype or Treg-depleted DREG mice receiving vehicle or MPO/BAY DCs. Wildtype (WT) or DREG mice were immunised with MPO/adjuvant (days 0 and 7). DREG mice received vehicle (saline; n = 4) or diphtheria toxin (DT; n = 6/group) on days 12-14 to deplete Tregs. Vehicle (saline) or BAY-treated MPO-pulsed DCs (MPO/BAY DCs) were administered to Treg-intact WT (saline, n = 3; DCs, n = 3) or DT-treated (Treg-depleted) DREG mice on day 14. CD4 and CD8 expression of IL-17A (MFI) and the proportion of IFNγ+ CD4 and CD8 T cells was assessed on day 18 by flow cytometry using lymph node cells re-stimulated with MPO for 48 hours. Data are presented as scatter plots with the mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
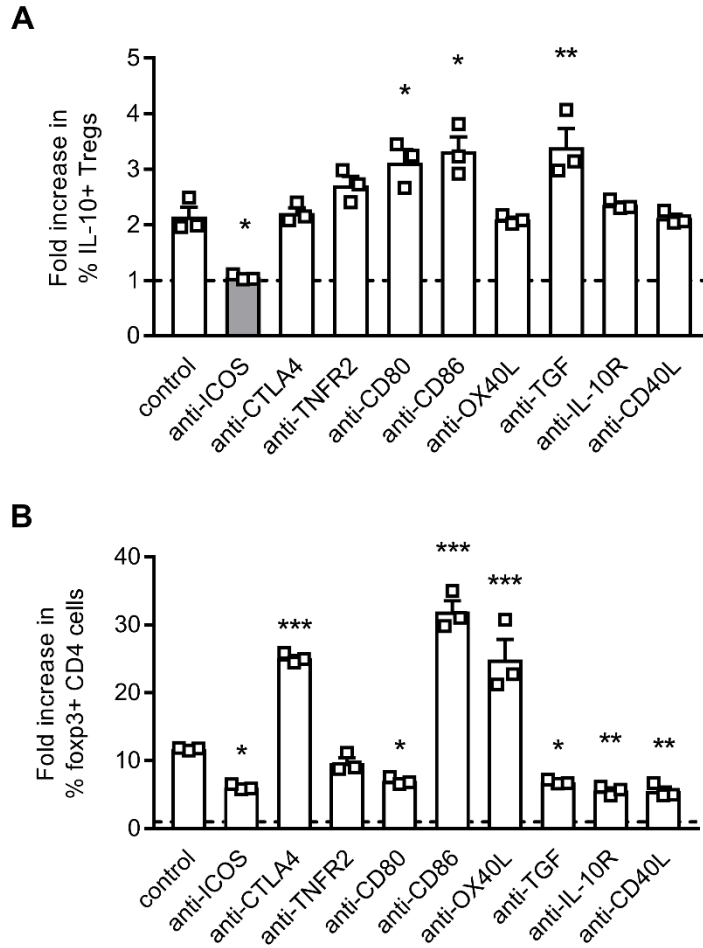
Supplemental Figure 4. The mechanisms by which MPO/BAY DCs regulate Treg responses. Tregs (CD4+foxp3+ cells) or CD4+foxp3- cells, isolated from MPO-immunised foxp3-GFP mice, were co-cultured with or without MPO/BAY DCs and LPS for 48 hours, in the presence of control IgG or blocking antibodies against ICOS, CTLA4, TNFR2, CD80, CD86, OX40L, TGFβ, IL-10R or CD40L. IL-10 expression by Tregs (in DC/Treg co-cultures) and the generation of CD4+foxp3+ from CD4+foxp3- cells (in DC/CD4+foxp3- co-cultures) was assessed by flow cytometry. (A) Fold increase in the percentage of IL-10+ Tregs by MPO/BAY DCs. (B) Fold increase in the percentage of newly generated CD4+foxp3+ cells by MPO/BAY DCs. The dotted lines represent fold increase of 1 (i.e. no increase in the percentage of IL-10+ cells and no increase in the percentage of CD4+foxp3+ cells). Data are presented as scatter plots with the mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (all P values are *versus* 'control').

Supplemental Figure 5. The effect of MPO/BAY DC-induced Tregs, in the presence or absence of an IL-10R antibody, on cytokine production by CD4 T cells. BAY-treated MPO-pulsed DCs (MPO/BAY DCs) were administered to MPO-immunised foxp3-GFP mice on day 14 and Tregs (CD4+foxp3/GFP+ cells) isolated on day 28. Then, vehicle (saline; n = 6/group) or BAY DC-induced Tregs (n = 6/group), and either control rat IgG or a blocking IL-10 receptor (IL-10R) antibody, were given to mice with established anti-MPO immunity (day 17). GN was triggered on day 21. On day 26, cytokine production by MPO-restimulated lymph node cells was measured by flow cytometry to determine the proportion of CD4 T cells expressing IL-17A, IFNγ or IL-4. Data is presented as scatter plots with the mean ± SEM * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.