Supplementary Figure Legends

**Supplementary Fig. 1 Kinetics of Treg depletion in tumor-bearing Foxp3-DTR mice.** Groups of BALB/c or C57BL/6 Fo

xp3-DTR mice (n=4-5/group) were injected s.c with 5 x 104 4T1.2 mammary carcinoma cells or 1 x 106 cells MC38 colon carcinoma cells respectively. When tumors reached a mean size of 40-50 mm2, mice were treated i.p with DT (250 or 500 ng/mouse) or PBS as indicated. Three and five days after treatment, spleens, blood, and tumors were collected and single cells suspensions generated. Gating on live CD45.2+ cells of lymphocyte morphology, the proportion of Foxp3+ Tregs within CD4+ TCRβ+ cells were determined in each organ. Data presented as mean ± SEM. Experiment was performed once.

**Supplementary Fig. 2 Development of blepharitis in tumor-bearing Foxp3-DTR mice after multiple DT treatment.** Groups of BALB/c or C57BL/6 Foxp3-DTR mice (n=4-5/group) were injected s.c with 5 x 104 4T1.2 mammary carcinoma cells or 1 x 106 cells MC38 colon carcinoma cells respectively. When tumors reached a mean size of 40-50 mm2, **(A)** BALB/c Foxp3-DTR mice were injected i.p. with 1 or 2 doses of DT (250 ng/mouse) or PBS 3 days apart while **(B)** C57BL/6 Foxp3-DTR mice were injected i.p with 1, 2 or 5 doses of DT (250 ng/mouse) four days apart. **(A)** BALB/c Foxp3-DTR mice were photographed 17 days after the first DT injectionwhile **(B)** C57BL/6 Foxp3-DTR mice were photographed 23 days after the first DT injection. PBS treated mice were photographed 15 days after injection. Arrows indicate the sites of blepharitis. Experiment was performed once.

**Supplementary Fig. 3 Prolonged Treg depletion induces** **severe irAEs in MC38 tumor-bearing mice.** Groups of C57BL/6 Foxp3-DTR mice were injected s.c with 1×106 MC38 colon carcinoma cells. When tumors reached a mean size of 40-50mm2, mice were treated with 1, 2 or 5 doses of DT (250 ng/mouse) or PBS given 4 days apart. **(A)** Following treatment, mice were weighed and changes to bodyweight during treatment period plotted. Data presented as mean ± SEM and significant differences between mice treated with 1 dose versus 5 doses of DT on day 29 post tumor inoculation determined by one-way ANOVA with Dunnet post-test analysis. **(B-C, F-K)** Organs and sera were collected either when tumor size reached maximum limit or when irAEs reached ethical limits, which necessitated euthanasia. **(B)** Spleen weights. Data presented as mean ± SEM; each symbol represents a single mouse. **(C)** RepresentativeH&E stained sections of spleen, ear, proximal colon, lung and liver (scale bar 200 m). **(D, E)** MC38 tumor-bearing mice were treated with 1 or 3 doses of DT (indicated as multiple DT) (250 ng/mouse) or PBS given every 4 days. Sera were collected 15 days after the first injection and IFNγand TNF levels determined. **(F)** Sera ANA with representative images (scale bar 33 µm) and **(G)** MFI values of each group shown. **(H, I)** Anti-dsDNA antibodies of both IgG and IgM isotypes in sera were determined by ELISA. **(J)** IgG deposition in glomeruli of frozen kidney sections was determined with representative images (scale bar 200 m) and **(K)** MFI values of each group shown. Data shown in **(D, E, J, K)** is from a single experiment (n=4-5/group) while data shown in **(B, F, H, I)** is pooled from 2 experiments (n=4-5/group). Significant differences between groups were determined by one-way ANOVA or two-way ANOVA respectively with Dunnet post test analysis comparing PBS- and DT-treated groups (\*p< 0.05, \*\*p< 0.01; \*\*\*p< 0.001, \*\*\*\*p< 0.0001).

**Supplementary Fig. 4 Transient Treg depletion or multiple anti-CTLA-4 and anti-PD-1 treatment induce similar biochemical autoimmunity in tumor-bearing mice.** Groups of BALB/c Foxp3-DTR mice (n=4-5/group) were injected s.c with 5 x 104 4T1.2 mammary carcinoma cells. Therapy commenced when tumors reached a mean size of 40-50 mm2. Groups of mice were treated i.p with 1 dose of DT (250 ng/mouse) and control Ig (500 g/mouse) or PBS and control Ig (500 g/mouse) or with anti-PD-1 and anti-CTLA-4 (all 250 g/mouse each) as indicated. Treatment with antibodies continued for 2 more doses given 3 days apart. One group of mice received 2 doses of DT given 3 days apart. 3 days after the final antibody treatment, organs were harvested. **(A)** Spleen weights of treated mice when culled. **(B)** Representative H&E stained sections of liver (scale bar 200 m). **(C)** Liver score of each group. **(D)** Sera anti-dsDNA antibodies of IgG isotype. Data expressed as mean ± SEM; each symbol represents a single mouse. Significant differences between PBS and treated groups were determined by one-way ANOVA with Dunnet post test analysis. (\*\*p < 0.01; \*\*\*p< 0.001, \*\*\*\*p< 0.0001). Experiment was performed once.

**Supplementary Fig. 5 Transient Treg depletion of tumor-bearing mice increases the expression of PD-1, TIM-3 and CD137 on T cells from spleen but not tumor. (A, B)** Groups of BALB/c or **(C, D)** C57BL/6 Foxp3-DTR mice (n=5/group) were injected s.c with 5 x 104 4T1.2 mammary carcinoma cells or 1 x 106 MC38 colon carcinoma cells respectively. When tumors reached a mean size of 40-50 mm2, mice were treated i.p with 1 dose of DT (250 ng/mouse) or PBS. 3 days later, spleen and tumor were harvested, single cell suspension generated and assessed by flow cytometry. Gating on live CD45.2+ cells of lymphocyte morphology, the expression of PD-1, TIM-3 and CD137 on CD4 T helper cells (CD4+ TCRβ+ Foxp3-), Tregs (CD4+ TCRβ+ Foxp3+) and CD8+ TCRβ+ T cells were determined. Data expressed as mean ± SEM. Significant differences between DT- and PBS-treated groups were determined by unpaired student t-test. (\*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001, \*\*\*\*p< 0.0001). Experiments were performed once.

**Supplementary Fig. 6 Transient** **Treg depletion in combination with anti-PD-1, anti-TIM-3 or anti-CD137 can all suppress established 4T1.2 or MC38 tumor growth.**  Fromthe same experiments as described and shown in Fig. 3A and C, data represented as mean tumor size ± SEM with the same data for PBS+control IgG, DT+control IgG and multiple DT-treated groups plotted on each graph for **(A-C)** 4T1.2 and **(D-F)** MC38 tumor-bearing mice. Significant differences in tumor size between DT+control IgG and DT+test antibody or PBS+control IgG and PBS+test antibody groups were determined by Mann-Whitney analysis (\*p< 0.05, \*\*p< 0.01). Data is representative of 2-3 experiments for both tumor models.

**Supplementary Fig. 7 Transient** **Treg depletion in combination with anti-PD-1, anti-TIM-3 or anti-CD137 differentially suppressed established CT26L5 tumor growth.** Groups of BALB/c Foxp3-DTR mice (n = 5/group) were injected s.c with 2 x 105 CT26L5 colon carcinoma cells. Therapy commenced when tumors reached a mean size of 40-50 mm2. Groups of mice were treated i.p with 1 dose of DT (250 ng/mouse) or PBS and 3 days later treated i.p with anti-PD-1, anti-TIM-3, anti-CD137 or control IgG (all 250 g/mouse) as indicated. Treatment with antibodies continued for 4 more doses given 3 days apart. One group of mice received 2 doses of DT given 3 days apart (indicated as multiple DT). **(A)** The tumor size of each individual mouse or **(B)** mean tumor size ± SEMare plotted. Data is representative of 2 independent experiments. Significant differences in tumor sizes between DT+control IgG and DT+test antibody groups were determined by Mann-Whitney analysis (\*\*p< 0.01).

**Supplementary Fig. 8 Transient** **Treg depletion in combination with anti-CD137 in tumor-bearing Foxp3-DTR mice induces severe irAEs.** From the same experiment as described and shown in Fig. 4. RepresentativeH&E stained sections of the indicated organs harvested from **(A)** 4T1.2 or **(E)** MC38tumor-bearing mice (scale bars 200 µM). **(B)** Sera ANA with MFI values of each group shown. **(C)** Sera anti-dsDNA antibodies of IgG isotype. From the same experiment as described and shown in **Fig. 3 A and C,** mice were weighed before and at different points during treatment with **(D, H)** mean weight change ± SEM for each time point plotted. Differences between DT+control IgG and all other groups determined by one-way ANOVA with Dunnet post test analysis. (\*p< 0.05, \*\*p < 0.01; \*\*\*p< 0.001, \*\*\*\*p< 0.0001). Data is representative of 2 independent experiments.

**Supplementary Fig. 9 Transient** **Treg depletion in combination with anti-CD137 increases the proportion of CD8+ T cells in multiple organs of tumor-bearing mice.** From the same experiment as described and shown in Fig. 5. Gating on live CD45.2+ cells of lymphocyte morphology, the proportion of CD8+ TCRβ+, CD4+ TCRβ+ Foxp3- and CD4+ TCRβ+ Foxp3+ T cells from the indicated organs were determined. Results shown are representative of 2 independent experiments. Significant differences between DT+control IgG and DT+test antibody treated groups were determined by one-way ANOVA with Dunnet post test analysis. (\*p < 0.05, \*\*p < 0.01; \*\*\*p< 0.001, \*\*\*\*p< 0.0001).

**Supplementary Fig. 10** **Transient** **Treg depletion in combination with anti-CD137 increases inflammatory cytokines in sera of tumor bearing mice.** (**A**) Groups of BALB/c or (**B**) C57BL/6 Foxp3-DTR mice (n=4-6/group) were injected s.c with 5 x 104 4T1.2 mammary carcinoma cells or 1 x 106 cells MC38 colon carcinoma cells respectively. Therapy commenced when tumors reached a mean size of 40-50 mm2. Groups of mice were treated i.p with DT (250 ng/mouse) or PBS and 3 days later treated i.p with anti-PD-1, anti-TIM-3, anti-CD137 or control IgG (all 250 g/mouse) as indicated. Treatment with antibodies continued for 2 more doses given 3 days apart. Some groups of mice received **(A)** 2 doses of DT given 3 days apart or **(B)** 3 doses of DT given 4 days apart (indicated as multiple DT). Three days after the final antibody treatment, sera IFNγ and TNF levels were measured. Data pooled from 2 independent experiments with significant differences between DT+control IgG and DT+test antibody groups determined by two-way ANOVA with Dunnet post test analysis.