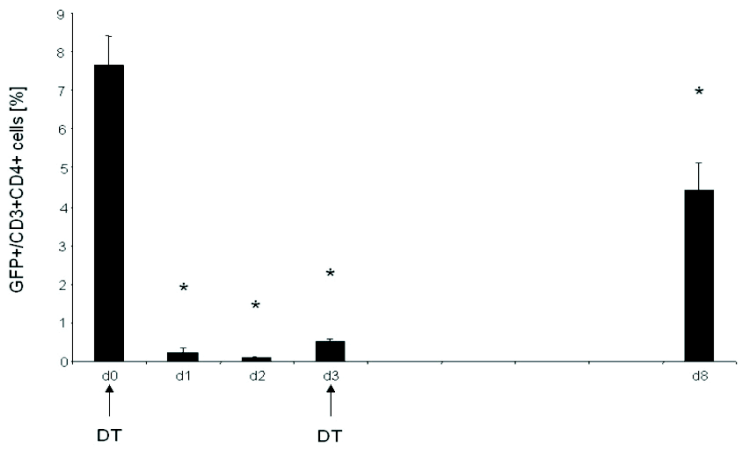
Selective depletion of Foxp3^{+} regulatory T cells promotes hypercholesterolemia and exacerbates experimental atherosclerosis

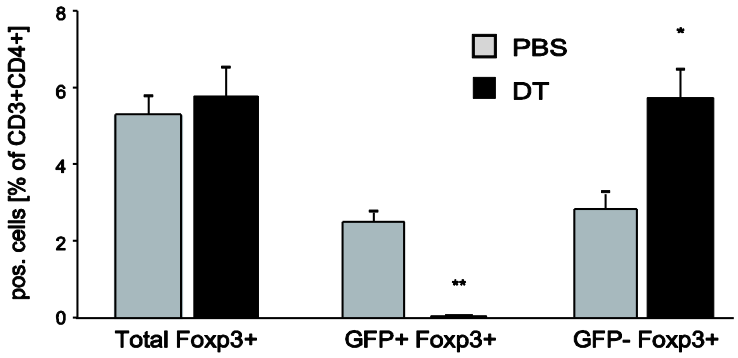
Roland Klingenberg, Norbert Gerdes, Robert M. Badeau, Anton Gisterå, Daniela Strodthoff, Daniel F. J. Ketelhuth, Anna M. Lundberg, Mats Rudling, Stefan K. Nilsson, Gunilla Olivecrona, Stefan Zoller, Christine Lohmann, Thomas F. Lüscher, Matti Jauhiainen, Tim Sparwasser, Göran K. Hansson

Supplemental Figures

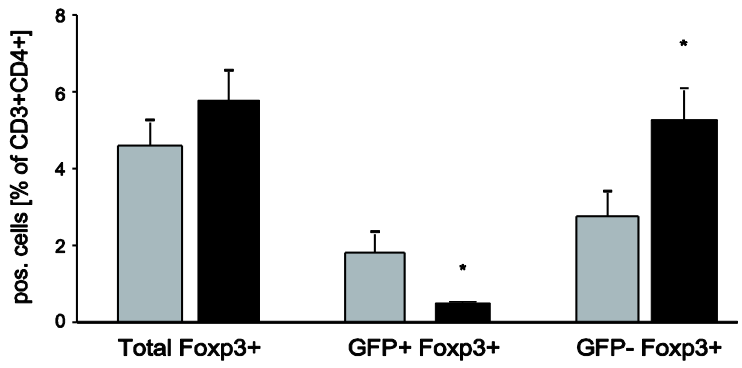
s1A


GFP+/CD3+CD4+ cells [%]
d0
d1
d2
d3
d8
DT
DT

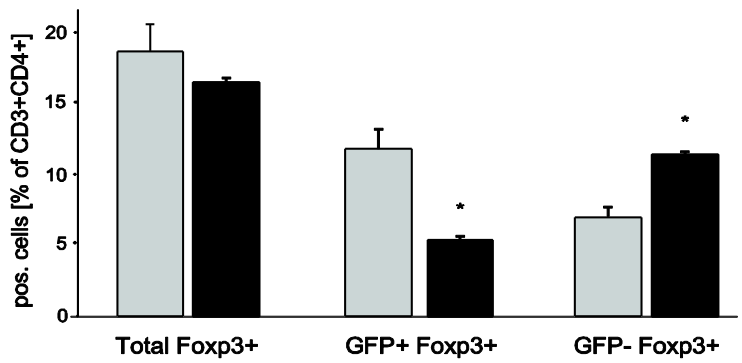
B


pos. cells [% of CD3+CD4+]
PBS
DT
Total Foxp3+
GFP+ Foxp3+
GFP- Foxp3+

C


pos. cells [% of CD3+CD4+]
Total Foxp3+
GFP+ Foxp3+
GFP- Foxp3+

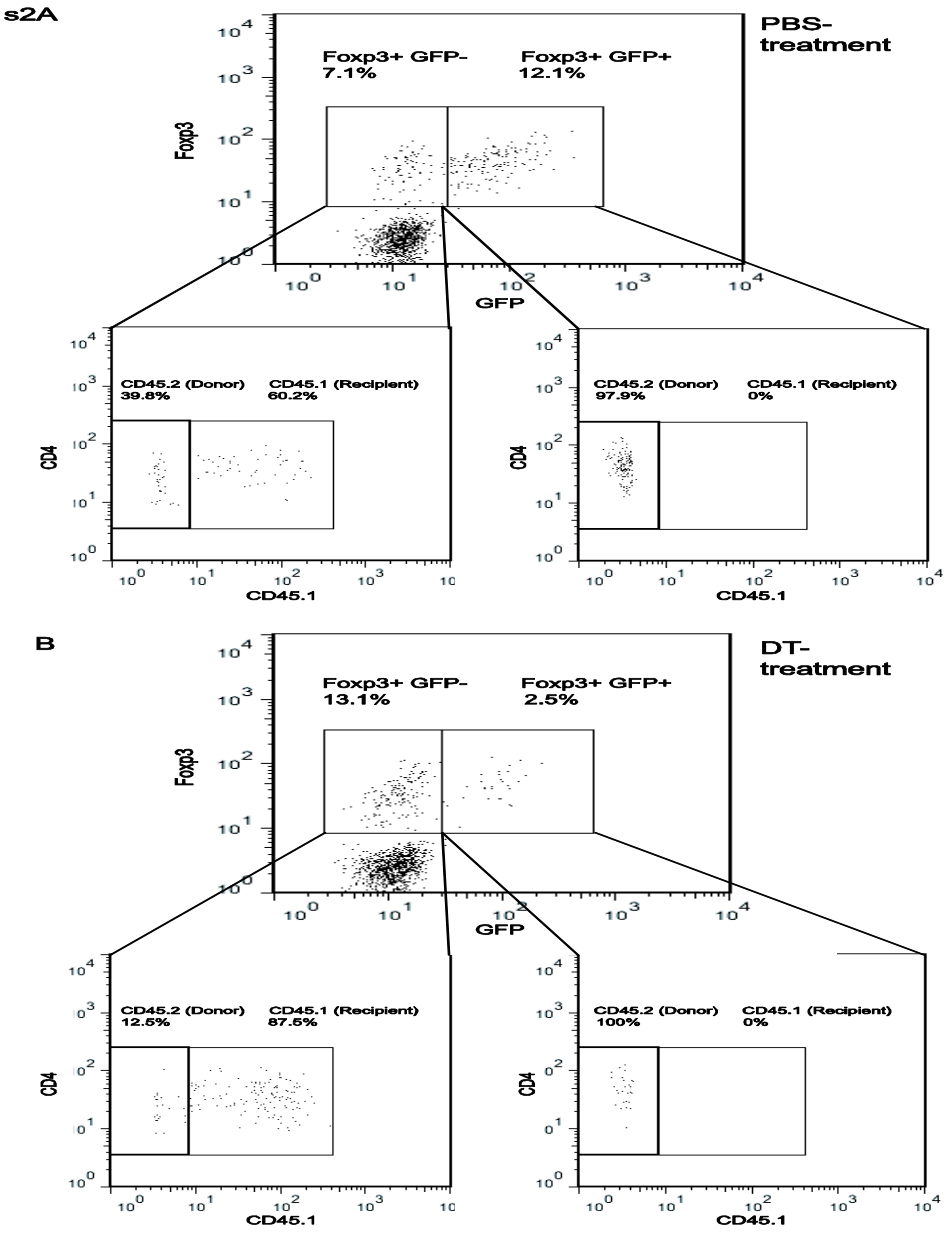
D


pos. cells [% of CD3+CD4+]
Total Foxp3+
GFP+ Foxp3+
GFP- Foxp3+

Supplemental Figure 1. **Dosing study and kinetics of transgenic Treg depletion in DEREG*/Ldlr*$^{-/-}$ bone marrow chimeras.**

(A) Pilot experiment: Kinetics of transgenic Treg depletion after DT injection. Percent eGFP^{+} cells in CD3^{+}CD4^{+} gate at different time points after one DT injection (FACS analysis). Diphtheria toxin (DT; 6.25 ng/kg body weight; i.p.) was administered to DEREG mice at the indicated time points. eGFP^{+} cells were quantified within the CD3^{+}CD4^{+} population by FACS analysis of peripheral blood. *$p<0.05$ vs d0; n=5.

(B-D) Depletion of eGFP-transgenic cells in relation to Foxp3^{+} within the CD3^{+}CD4^{+} population in peripheral blood of DEREG*/Ldlr*$^{-/-}$ bone marrow chimeras treated with DT or PBS drawn at 10 days (B), 30 days (C) and 60 days (D). Data are generated from CD3^{+}CD4^{+} cells (n = 7-8 mice per group) using FACS analysis.



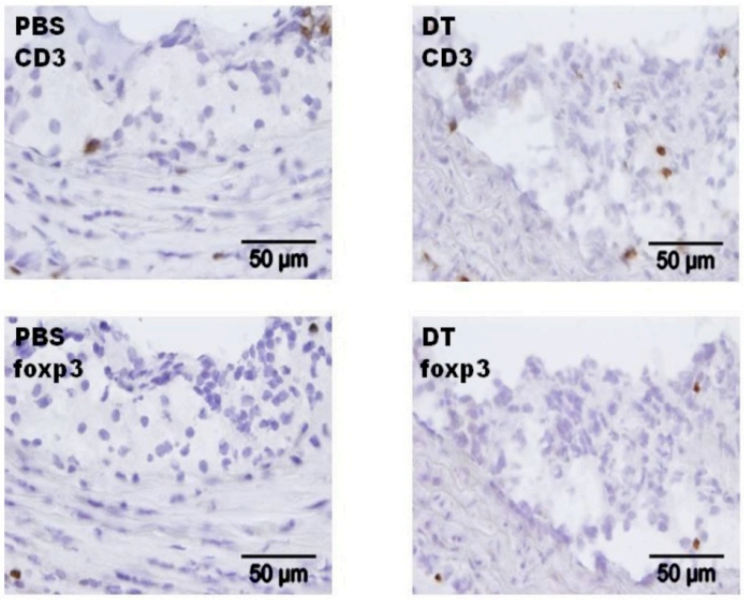
Supplemental Figure 2. **Regeneration of GFP$^-$ Foxp3$^+$ Treg from host pool.**

CD45.2$^+$ bone marrow was transplanted into *Ldlr*$^{-/-}$ mice carrying the CD45.1 surface marker.

(A) Representative FACS plot of spleen cells from a PBS-treated chimeric mouse. The majority of Foxp3$^+$ cells are GFP$^+$ derived from CD45.2$^+$ donor bone marrow.

(B) Representative FACS plot of spleen cells from a DT-treated chimeric mouse. Most of the Foxp3$^+$ GFP$^+$ cells have disappeared in DT-treated animals and the remaining Foxp3$^+$ cells are GFP$^-$ derived from the CD45.1$^+$ recipient population.

s3A



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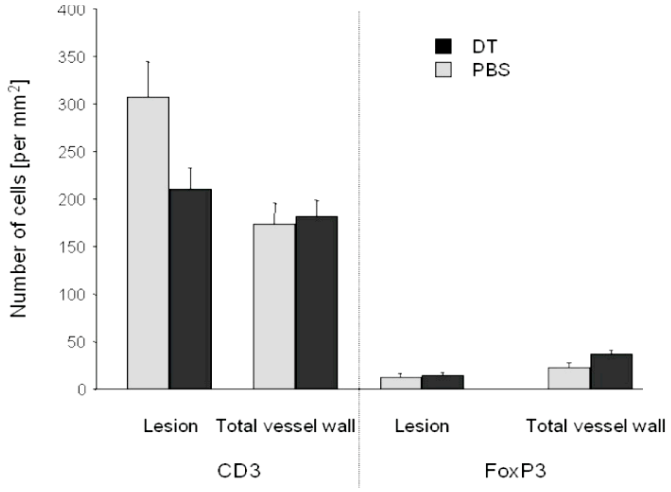
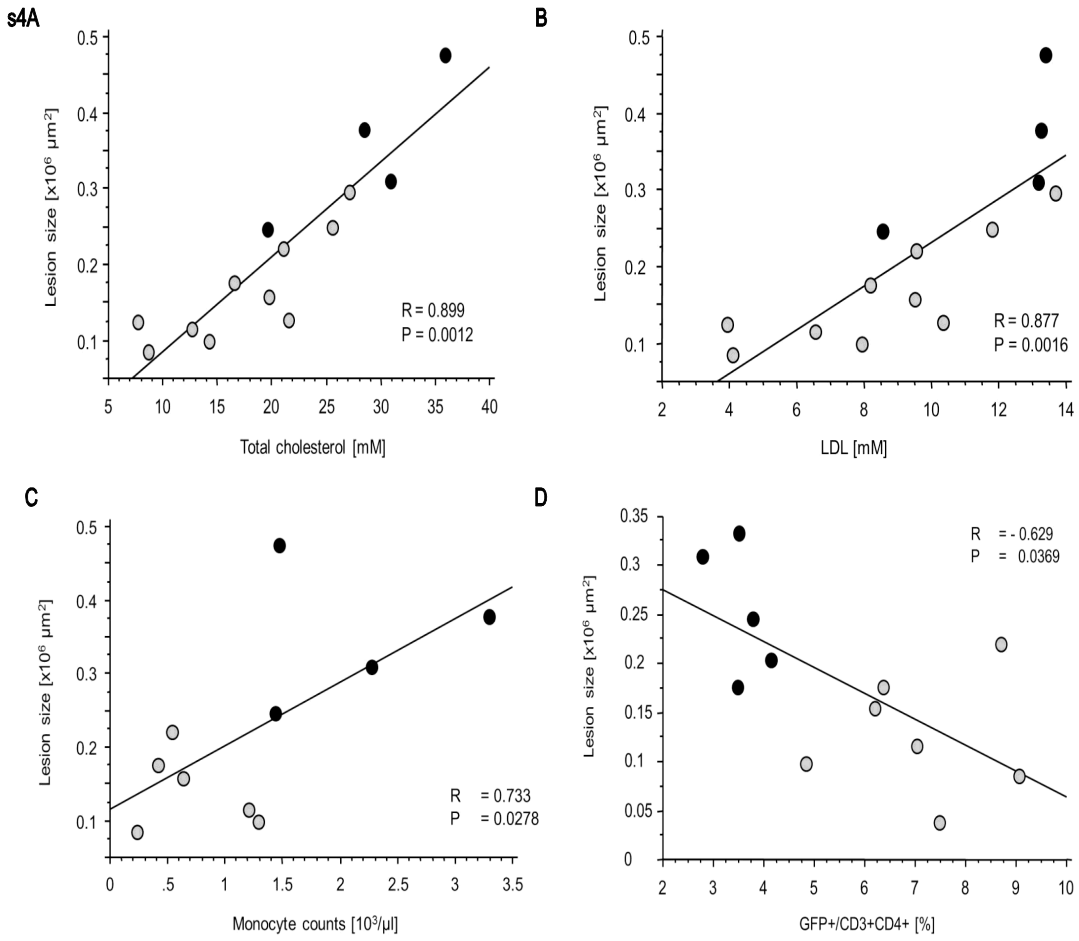


Supplemental Figure 3. **T cells including Treg are present in atheroma and vascular wall.**

(A) Representative micrographs of immunohistochemical staining of the aortic sinus of DEREG/*Ldlr*$^{-/-}$ chimeric mice treated with either PBS or DT and fed an atherogenic diet for 8 weeks. Sections were stained for total T cells (CD3) and Treg (Foxp3).

(B) Quantitative analysis of immunostaining for CD3 and Foxp3 in lesions and total vessel wall. Data show stained cells per cross-section area. N=12 (PBS) and n=8 (DT), respectively.

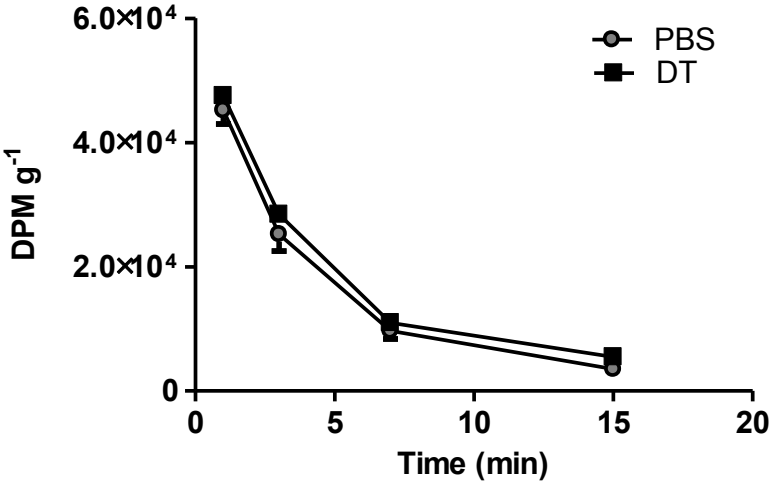


Supplemental Figure 4. **Correlations with lesion size in aorta.**

Distribution of values for individual animals are shown.

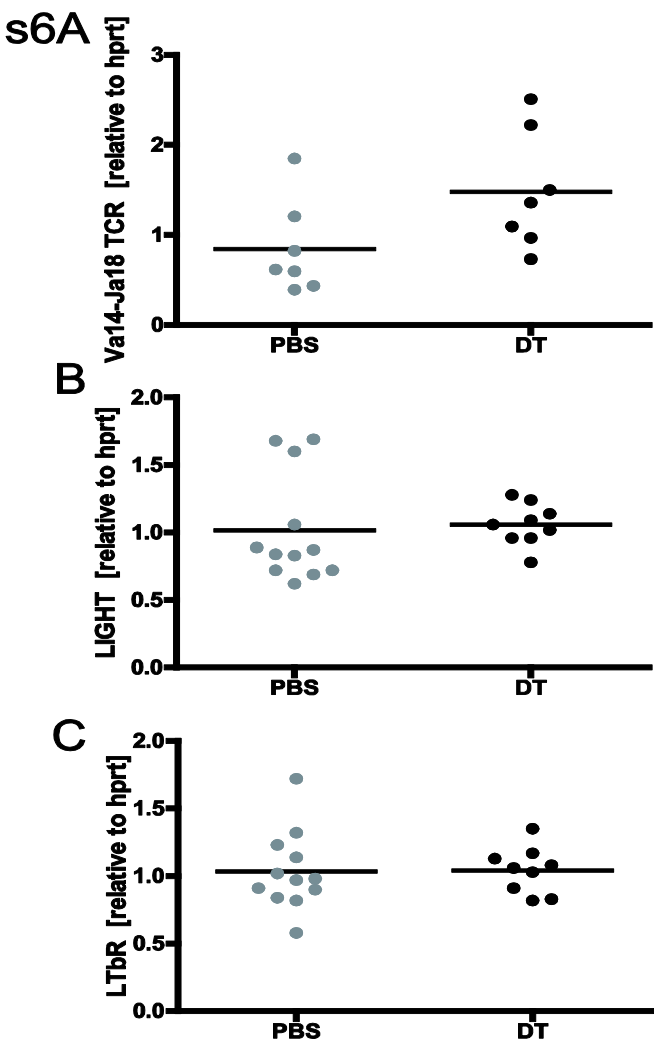
(A) Lesion size vs. total plasma cholesterol (mM), (B) plasma LDL cholesterol (mM), (C) blood monocyte count (cells x 10^3/µl) and (D) $eGFP^+$ Treg (percent of all $CD3^+CD4^+$ cells in spleen). DT, black circles, PBS, grey circles.

S 5



Supplemental Figure 5. Kinetics of chylomicron [^{3}H]-triglyceride clearance from blood.

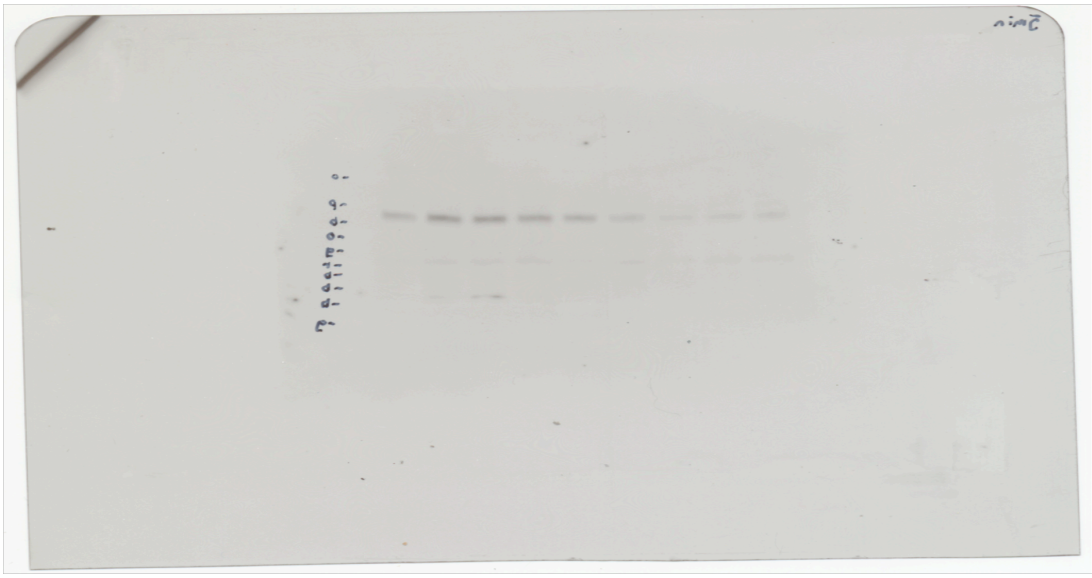
In vivo turnover of [^{3}H]triglyceride/[^{14}C]retinol chylomicron particles in chimeric DEREG/*Ldlr*$^{-/-}$ mice treated for 8 weeks with PBS or DT. Radioactivity was measured in the [^{3}H] channel at the indicated time points. DPM, disintegrations per minute. N= 6 PBS; n=8 DT. Mean ± S.E.M. are shown.



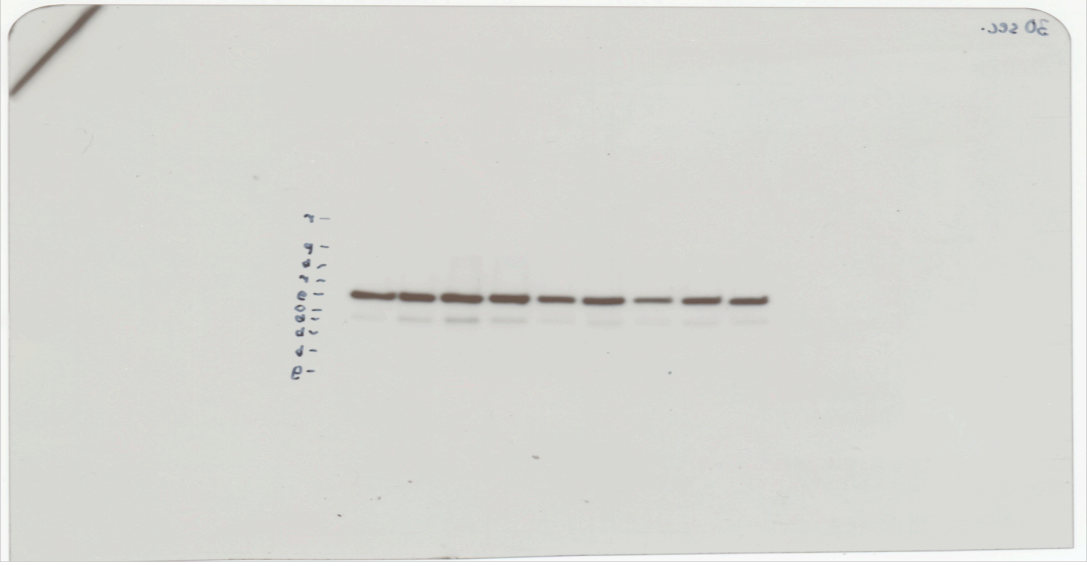
Supplemental Figure 6. Treg depletion did not affect the LIGHT/NKT/lipase pathway.

Quantitative real-time RT-PCR analysis of liver mRNA from DEREG/*Ldlr*$^{-/-}$ chimeric mice treated for 8 weeks with DT or PBS. Transcript levels were normalized to HPRT. *p<0.05. N=6 per group. (A) T cell receptor Vα14-Jα18 specific for iNKT cells; (B) LIGHT (TNFSF14), (C) Lymphotoxin-βreceptor. Hepatic lipase mRNA is shown in Figure 7 of the core manuscript.

S 7A



S 7B



Supplemental Figure 7. Hepatic expression of sortilin-1 is significantly decreased upon Treg depletion.

Full uncut immunoblots of liver tissue stained for sortilin-1 (A) and tubulin (B). The left 5 lines show PBS treated control mice, the following 4 lanes DT treated ones. Annotations are provided in Figure 8.