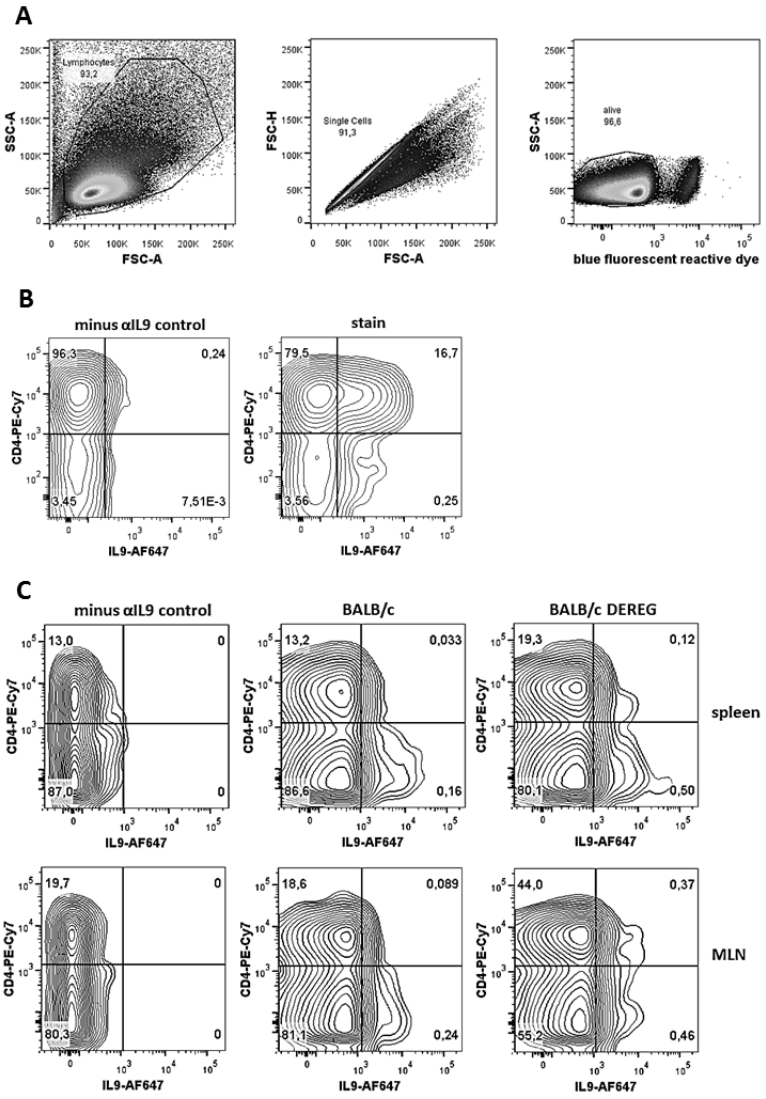
Figure S5: Intracellular IL-9 staining

S5A: Representative gating strategy for exclusion of doublets and dead cells is shown.
S5B: Representative plots for identification of IL-9$^+$ CD4$^+$ cells in Th9 polarized spleen cell cultures.
S5C: Representative plots for identification of IL-9$^+$ CD4$^+$ and CD4$^-$ cells in spleen and MLN from *S. ratti* infected and DT treated BALB/c and BALB/c DEREG mice.



Th9 polarization:
For Th9 polarization T cells were isolated from spleens using CD4$^+$ T cell Isolation Kit II (MACS Miltenyi Biotec) according to the manufacturers recommendation. 1x10^7 CD4$^+$ T cells were cultured in 10 mL RPMI medium on purified anti-mouse CD3ε (clone 145-2C11) coated cell culture dishes in the presence of 5 µg/mL purified anti-mouse CD28 (clone 37.51), 10 µg/mL purified anti-mouse IFN-γ (clone AN-18), 20 ng/mL recIL-2, 20 ng/mL recIL-4, 10 ng/mL human TGF-β (all Biolegend) for 3 days at 37°C and 5% CO_2.

Detection of *S. ratti*-induced IL-9 producing cells:
To detect IL-9 producing cells ex vivo BALB/c and BALB/c DEREG mice were infected s.c. with 2000 iL3 *S. ratti*. Mice received 0,5 µg DT on three consecutive days starting one day before infection. Mice were sacrificed day 6 p.i and reduced parasite burden in the small intestine of Treg depleted BALB/c DEREG mice compared to non depleted BALB/c mice was verified (data not shown). Spleens and mesenteric lymph nodes (MLN) were prepared, stimulated and analyzed for IL-9 production.

Intracellular IL-9 staining:
2-3x10^6 cells were incubated for 6 h with 500 ng/mL PMA (Phorbol-12-myristat-13-acetat, Sigma) and 500 ng/mL Ionomycin (Sigma) in the presence of 1x Brefeldin A Solution (Biolegend) at 37°C and 5% CO_2. Cells were first stained with LIVE/DEAD Fixable Blue Dead Cell Stain (1:1000, Invitrogen) in PBS for 30 min at 4°C. Cells were washed and subsequently surface stained with PE-Cy7-labeled anti-CD4 (clone RM4-5, Biolegend) for 30 min at 4°C. Cells were fixed and permeabilized using Foxp3-Fixation/Permeabilization Kit (eBioscience) according to the manufacturers recommendation. Intracellular IL-9 was detected using Alexa Fluor 647-labeled anti-mouse IL-9 (clone RM9A4, Biolegend) for 30 min at room temperature. Cells were washed and analyzed on a LSRII.