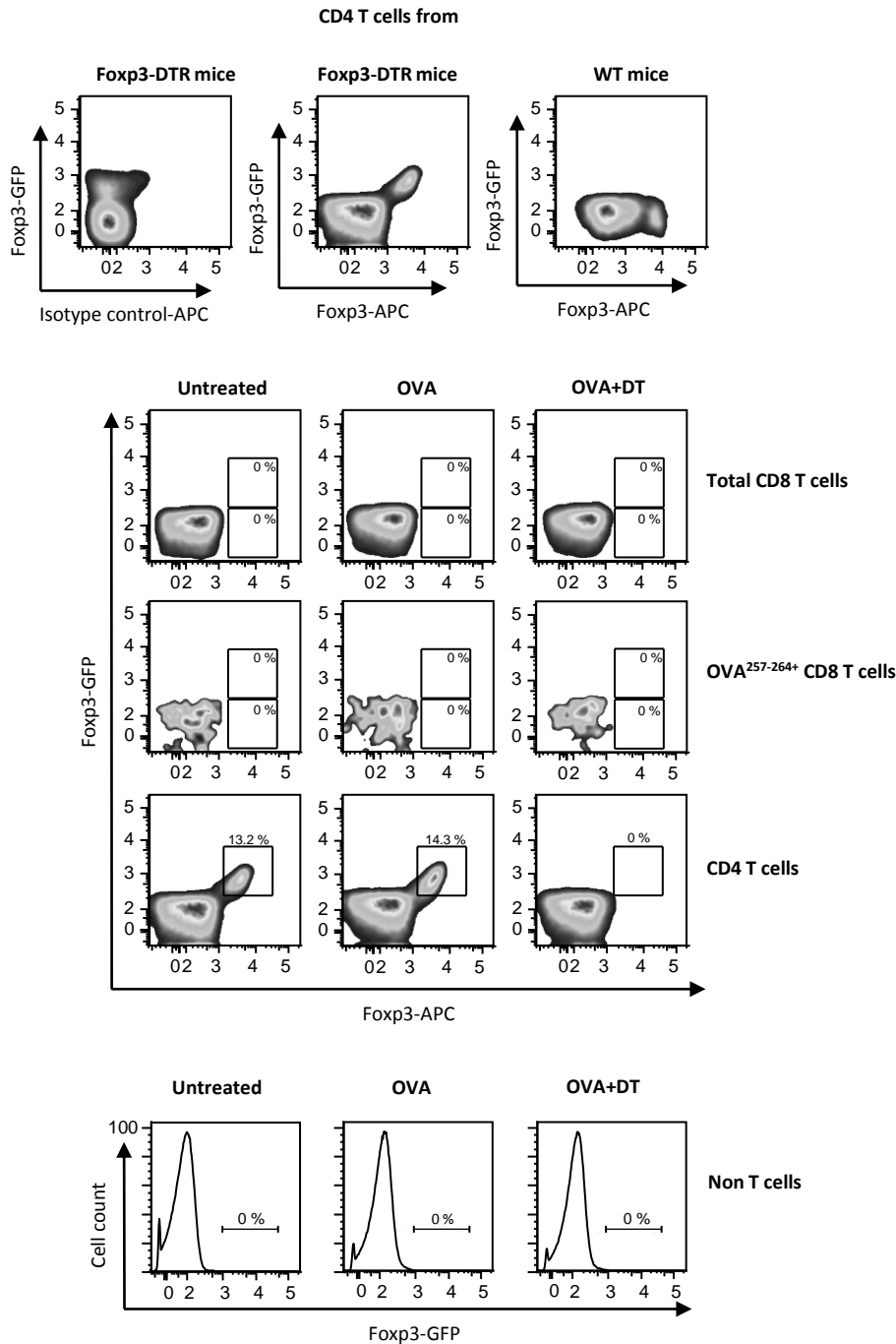
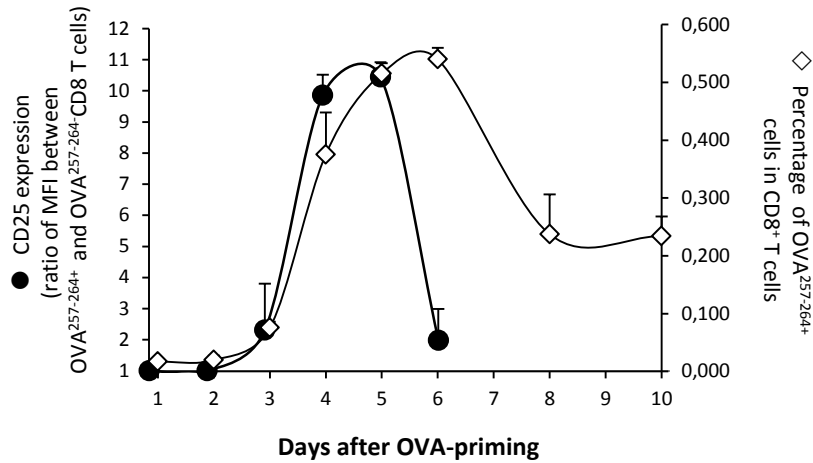


Supplementary Figure S1



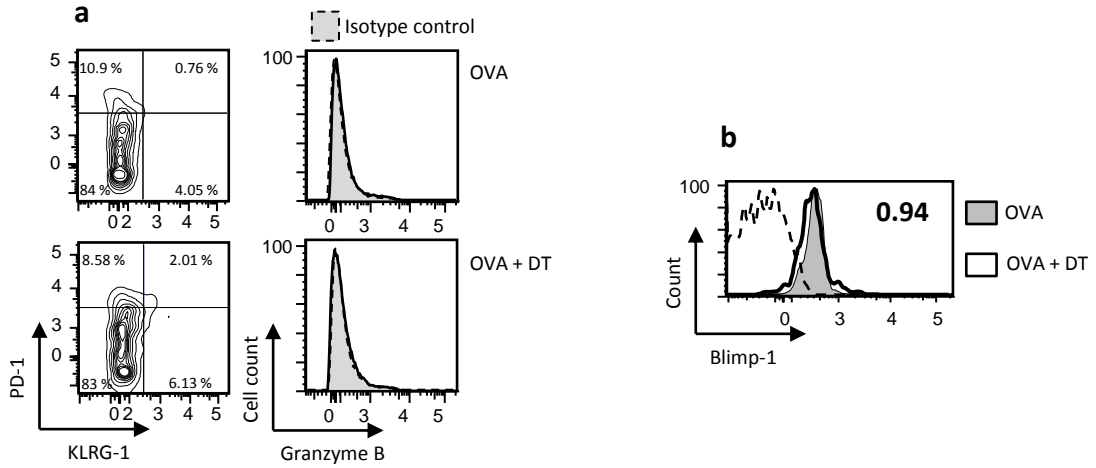
Supplementary figure S1: CD8 T cells do not express FoxP3 either at rest or following antigen activation. Foxp3-DTR mice were injected i.v. with 10^4 OT-I cells. Two days later, mice were vaccinated with OVA or left untreated. Some mice received 2 injections of DT at day 3 and 5 (OVA + DT). On day 6, splenocytes were stained with anti-CD3, anti-CD4, anti-CD8, dextramer OVA²⁵⁷⁻²⁶⁴-PE and anti-Foxp3-APC and analyzed by flow cytometry. Foxp3-expressing endogenous cells should be Foxp3-GFP⁺ and Foxp3-APC⁺ while exogenous OT-I cells, if Foxp3⁺, should be Foxp3-GFP⁻ but Foxp3-APC⁺.

Supplementary Figure S2



Supplementary Figure S2: Kinetics of activation and expansion of specific CD8 T cells following OVA priming. Foxp3-DTR mice were injected i.v. with 10^4 OT-I cells. Two days later, the mice were vaccinated with OVA. CD25 expression on OVA²⁵⁷⁻²⁶⁴-specific CD8 T cells was analyzed by flow cytometry daily from day 1 to day 6. Results are expressed as the CD25 MFI ratio between OVA²⁵⁷⁻²⁶⁴-specific CD8 T cells and OVA²⁵⁷⁻²⁶⁴-non specific CD8 T cells (closed circles). The percentage of OVA²⁵⁷⁻²⁶⁴-specific CD8 T cells among total CD8 T cells is also shown up to day 10 following OVA vaccination (open diamonds). Results are means \pm SD of data from 2 independent experiments with 3 mice per time-point.

Supplementary Figure S3



Supplementary Figure S3: Expression of KLRG-1 and Blimp-1 in resting memory CD8 T cells generated in the presence or absence of Tregs. Foxp3-DTR mice were injected i.v. with 10^4 OT-I cells. Two days later, the mice were vaccinated with OVA. Some mice received 2 injections of DT at day 3 and 5 (OVA + DT). Fifty days later, memory CD44⁺ OVA²⁵⁷⁻²⁶⁴-specific CD8 T cells were analyzed by flow cytometry for KLRG-1, PD-1 and Granzyme B (**Supplementary Fig. S3a**) and Blimp-1 (**Supplementary Fig. S3b**) expression. In **Fig. S3b**, the number in the top right corner represents the MFI ratio between the OVA+DT and OVA conditions and was calculated as follows: $[(\text{MFI OVA + DT} - \text{MFI isotype control}) / (\text{MFI OVA} - \text{MFI isotype control})]$. Dashed lines represent the isotype control. Results shown in Fig. S3a and S3b are representative of 3 independent experiments involving 2-3 mice per condition per experiment.