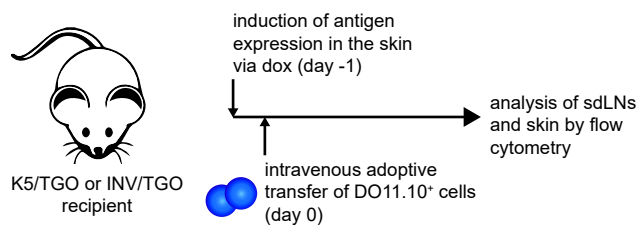
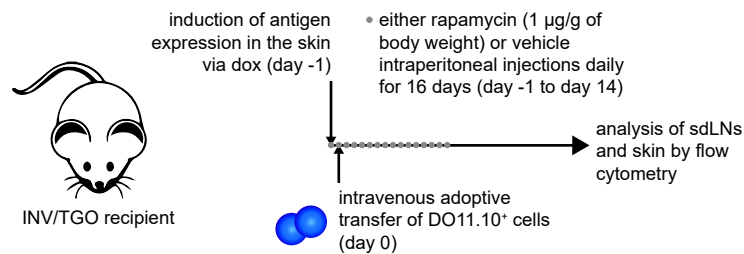


Supplemental figure 1

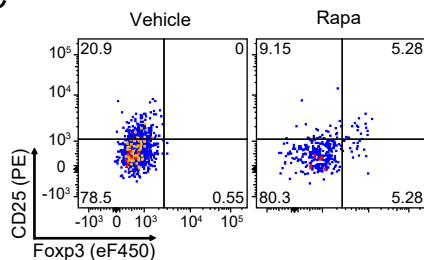
A



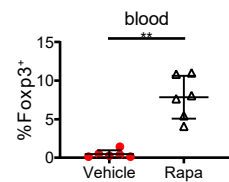
B



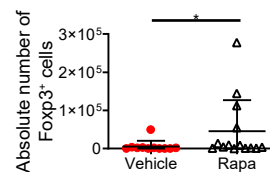
C



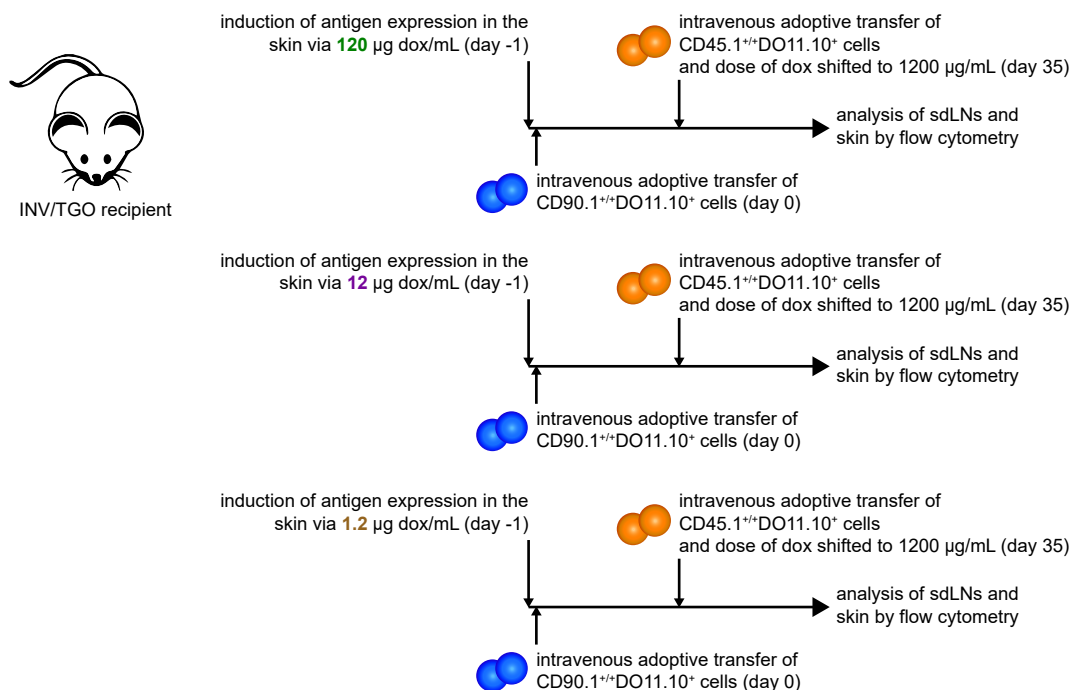
D



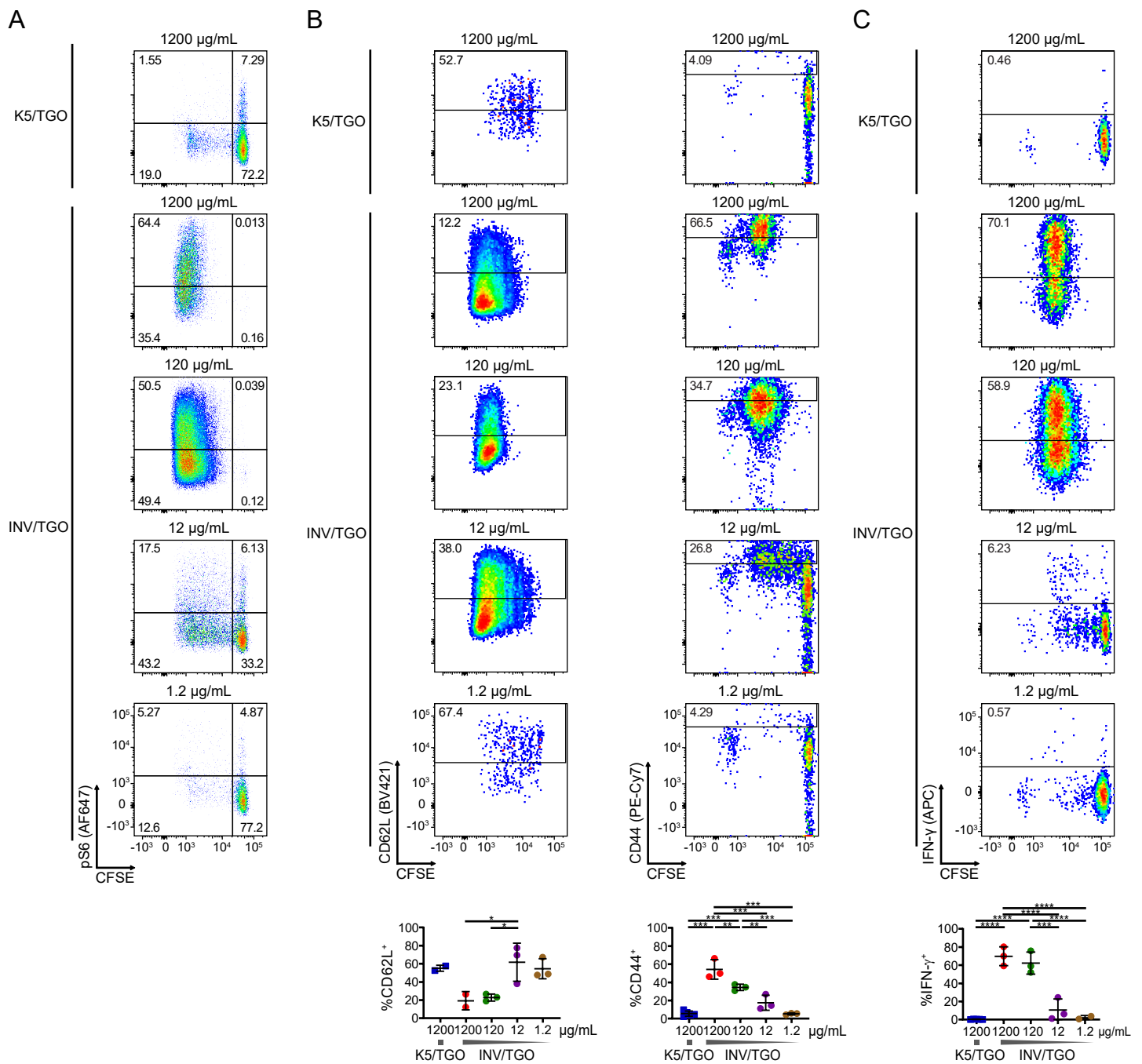
E



F



Supplemental Figure 1: Experimental schemes and the modulation of S6 phosphorylation impacts peripheral Foxp3⁺ cell generation. (A) Scheme of experimental set up for Fig. 1. (B) Scheme of experimental set up for Fig. 2. (C, D and E) OVA expression in the skin was induced by 1200 µg dox/mL a day before the transfer of 0.5-0.9x10⁶ LN cells from DO11.10 Rag2^{-/-} mice into INV/TGO mice. Starting a day before adoptive transfer mice were treated with either rapamycin (1µg/g of body weight) or vehicle i.p. injections daily for 16 days. (C) 47 days after adoptive transfer, blood was taken by vena saphena bleeding, red blood cells were lysed and the expressions of Foxp3 and CD25 by DO11.10 cells were analyzed by flow cytometry. Representative flow plots of live gated CD4⁺ DO11-TCR⁺ T cells. (D) As (C), graphical summary of the percentages of Foxp3⁺ DO11.10 cells in the blood on day 47 (N =6/group). (E) Absolute number of recovered Foxp3⁺ DO11.10 cells from sdLNs between days 41-67 (vehicle) and 71-89 (rapa). Cumulative data from 4 experiments, N ≥ 11/group. (F) Scheme of experimental set up for Fig. 4C-E. Mean and SD is shown.



Supplemental Figure 2: T cell activation and differentiation depends on dose of tissue antigen in INV/TGO mice. OVA expression in the skin was induced by the indicated doses of dox (1200 – 1.2 µg/mL) and $0.9 \cdot 2 \times 10^6$ CFSE-labeled LN cells from DO11.10 Rag2^{-/-} mice were transferred into K5/TGO or INV/TGO mice. sdLNs were isolated 2-4 days after transfer and single-cell suspensions analyzed by flow cytometry either with immediate fixation ex vivo (A) or without fixation (B, C). (A) Representative flow plots of pS6 expression in DO11.10 cells. (B) Representative flow plots and percentages of CD62L⁺ (left) and CD44⁺ (right) DO11.10 cells (N ≥ 2/group). (C) Representative flow plots and percentages of IFN-γ producing DO11.10 cells (representative of 2 experiments, N ≥ 3/group in total). Mean and SD is shown.

Table S1: Detailed list of antibodies and reagents

Mouse treatment		
Reagent	Company	Catalog number
doxycycline	Sigma-Aldrich	D9891
rapamycin	Selleckchem	S1039
Cell culture medium		
Reagent	Company	Catalog number
RPMI 1640	Gibco	31870074
Fetal Bovine Serum (FBS)	Thermo Fisher	10500064
Penicillin/streptomycin	Sigma-Aldrich	P0781
L-Glutamine	Gibco	A2916801
NEAA	Gibco	11140035
Sodium-Pyruvate	Sigma-Aldrich	S8636
2-Mercaptoethanol	Gibco	31350-010
Skin digestion		
Reagent	Company	Catalog number
Collagenase XI from Clostridium histolyticum	Sigma-Aldrich	C9407
Hyaluronidase	Sigma-Aldrich	H3506
DNase	Sigma-Aldrich	DN25
Cellular activation		
Reagent	Company	Catalog number
PMA	Sigma-Aldrich	P8139
Ionomycin	Sigma-Aldrich	I06434
Brefeldin A	Sigma-Aldrich	B7651
Flow cytometry		
Reagent	Company	Catalog number
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A7030
DPBS	Gibco	14190169
Foxp3 / Transcription Factor Staining Buffer Set	eBioscience	00-5523-00
Methanol	Sigma-Aldrich	322415
Paraformaldehyde	Electron Microscopy Sciences	15710
Pharm Lyse	BD	555899
Anti-mouse CD152 APC (UC10-4B9)	eBioscience	17-1522-82
Anti-mouse CD25 PE (PC61.5)	eBioscience	12-0251-82
Anti-mouse CD3 eFluor 450 (17A2)	eBioscience	48-0032-82
Anti-mouse CD357 PE (DTA-1)	Tonbo biosciences	50-5874-U100
Anti-mouse CD4 APC-eFluor 780 (GK1.5)	eBioscience	47-0041-82
Anti-mouse CD4 APC-eFluor 780 (OKT4)	eBioscience	47-0048-42
Anti-mouse CD4 FITC (GK1.5)	eBioscience	11-0041-82
Anti-mouse CD4 V500 (RM4-5)	BD	560782
Anti-mouse CD44 FITC (IM7)	eBioscience	11-0441-82
Anti-mouse CD45.1 APC-eFluor 780 (A20)	eBioscience	47-0453-82
Anti-mouse CD45.1 FITC (A20)	eBioscience	11-0453-82
Anti-mouse CD62L APC-eFluor 780 (MEL-14)	eBioscience	47-0621-82
Anti-mouse CD62L BV421 (MEL-14)	BD	562910
Anti-mouse CD90.1 APC-eFluor 780 (HIS51)	eBioscience	47-0900-82
Anti-mouse CD90.1 FITC (OX-7)	BD	554897
Anti-mouse CD90.1 PerCP (OX-7)	BD	557266
Anti-mouse DO11.10 TCR biotin (KJ1-26)	eBioscience	13-5808-82
Anti-mouse DO11.10 TCR FITC (KJ1-26)	eBioscience	11-5808-82
Anti-mouse DO11.10 TCR PE (KJ1-26)	eBioscience	12-5808-82
Anti-mouse DO11.10 TCR PerCP-eFluor 710 (KJ1-26)	eBioscience	46-5808-82
Anti-mouse FOXP3 eFluor 450 (FJK-16s)	eBioscience	48-5773-82
Anti-mouse FOXP3 PE (NRRF-30)	eBioscience	12-4771-82
Anti-mouse IFN- γ APC (XMG1.2)	eBioscience	17-7311-82
Anti-mouse IL-2 PE (JES6-5H4)	eBioscience	12-7021-82
Anti-mouse TNF- α PE-Cy7 (MP6-XT22)	eBioscience	25-7321-82
Carboxyfluorescein succinimidyl ester (CFSE)	eBioscience	65-0850-84
Fixable Viability Dye eFluor 506	eBioscience	65-0866-14
Fixable Viability Dye eFluor 520	eBioscience	65-0867-18
Fixable Viability Dye eFluor 780	eBioscience	65-0865-14
Goat anti-Rabbit IgG (H+L) Alexa Fluor 647	Invitrogen	A-21245
Phospho-S6 Ribosomal Protein (Ser235/236) (2F9)	Cell Signaling Technology	4856
Streptavidin PE-Cy7	eBioscience	25-4317-82
RNA isolation and RT-PCR		
Reagent	Company	Catalog number
gDNA eliminator column	Quiagen	74134
iScript TM reverse transcription super mix	Bio-Rad	1708841
GoTaq qPCR master mix 2X	Promega	A600