Supplementary Information

The transcription factor Foxp1 is a critical negative regulator of T follicular helper cell differentiation

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Supplementary Figures 1-11

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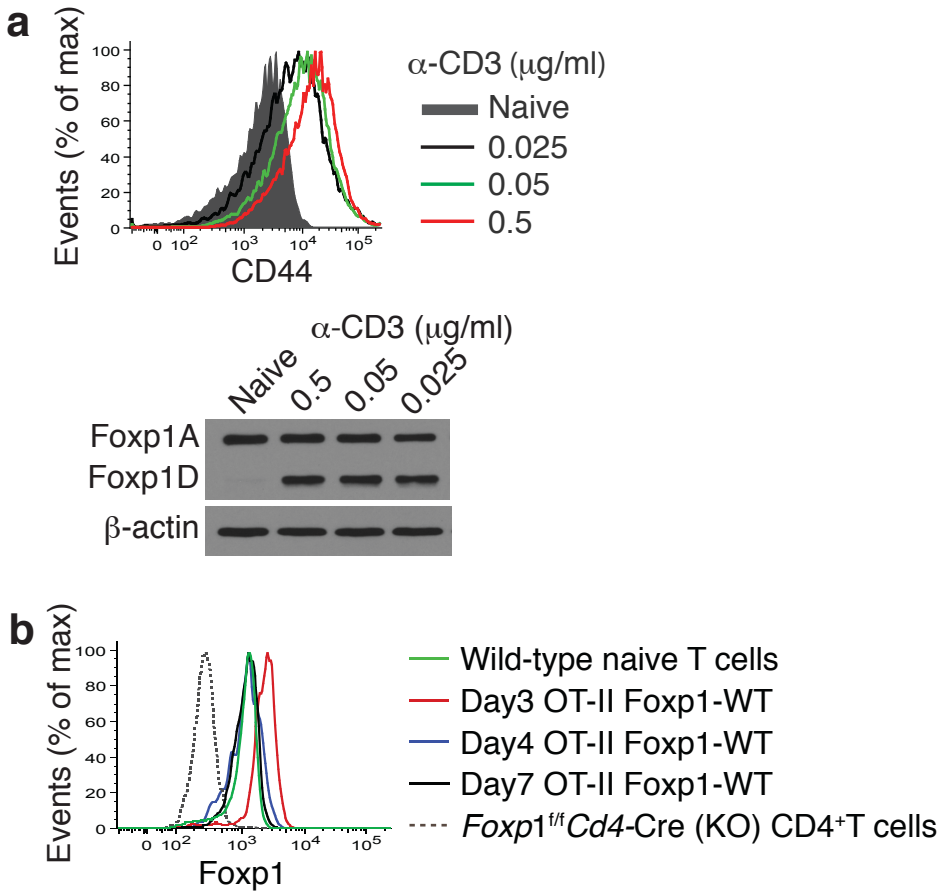
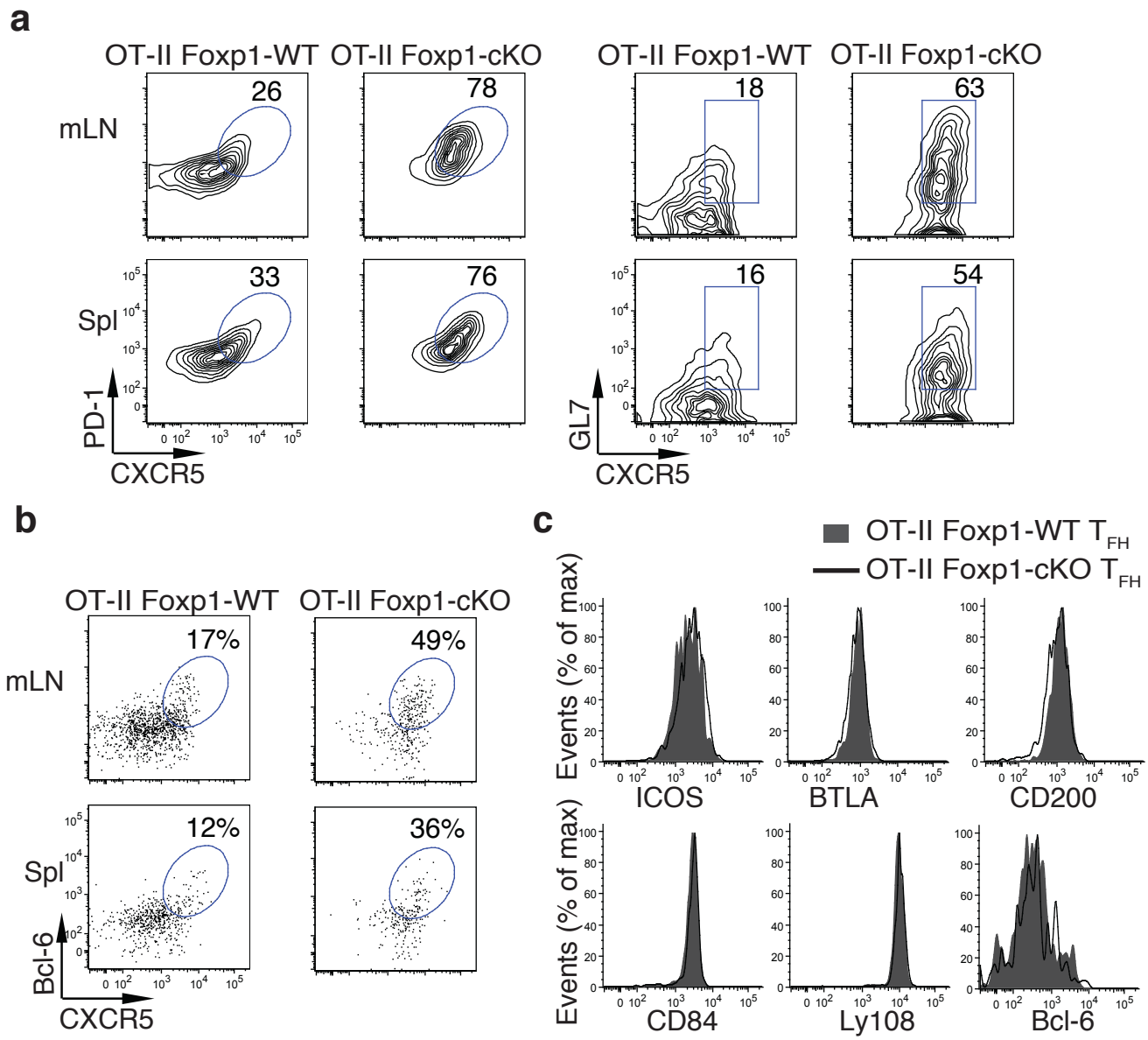
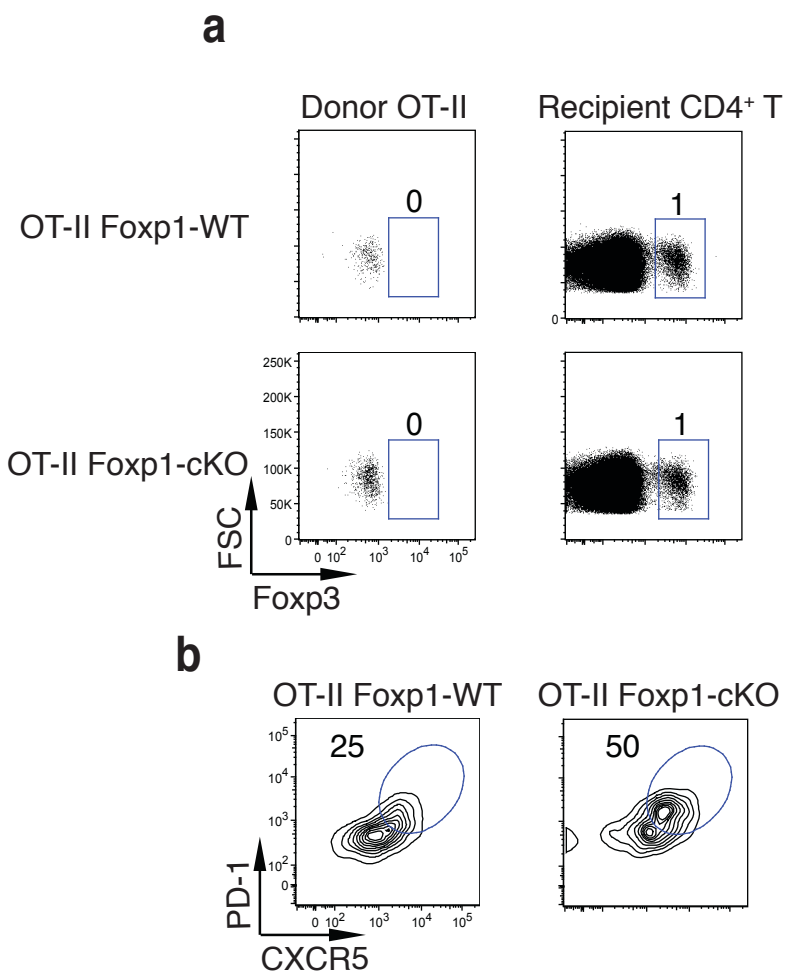


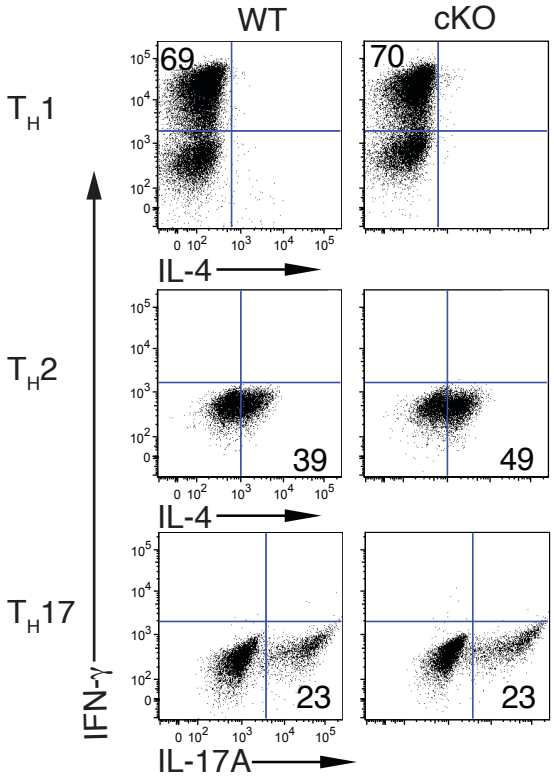
Supplementary Fig. 1. Foxp1D induction by TCR stimulation *in vitro* and *in vivo*. (**a**) Wild-type naive CD4$^+$ T cells were stimulated by plate-bound anti-CD3 antibodies at different concentrations for 48 hours, then analyzed by immunoblotting for Foxp1 expression and by staining for cell surface markers CD44. (**b**) Wild-type naive OT-II T cells were transferred into Ly5.1$^+$ C57BL/6 recipient mice followed by immunization with NP-OVA in alum. Foxp1 expression was analyzed by intracellular staining in donor OT-II T cells at different time points after immunization. *Foxp1*$^{f/f}$*Cd4*-Cre (KO) CD4$^+$ T cells were used as controls. Results represent three (**a**) and two (**b**) independent experiments.



Supplementary Fig. 2. T_{FH} cell differentiation of Foxp1-deficient OT-II T cells in Ly5.1^{+} C57BL/6 recipient mice. Purified naive OT-II Foxp1-WT or OT-II Foxp1-cKO T cells were transferred into Ly5.1^{+} C57BL/6 recipient mice followed by immunization with NP-OVA in alum. (**a**) Percentages of PD-1^{hi}CXCR5^{hi} T_{FH} cells and GL7^{hi}CXCR5^{hi} GC T_{FH} cells in splenic donor OT-II T cells 6 days after immunization. (**b**) Bcl-6 expression in donor OT-II T_{FH} cells by intracellular staining 6 days after immunization. (**c**) Phenotypes of donor OT-II T_{FH} cells by cell surface staining 6 days after immunization. Results represent at least two independent experiments.



Supplementary Fig. 3. The effect of Foxp1 on T_{FH} differentiation is cell-intrinsic. (**a**) Purified naive OT-II Foxp1-WT or OT-II Foxp1-cKO T cells were transferred into Ly5.1$^+$ SMARTA recipient mice followed by immunization with NP-OVA in alum. Foxp3 expression in splenic donor OT-II T cells and recipient CD4$^+$ T cells in SMARTA recipient mice were analyzed 6 days after immunization. (**b**) Naive OT-II Foxp1-WT and OT-II Foxp1-cKO T cells were mixed at 1:1 ratio and co-transferred into Ly5.1$^+$ C57BL/6 recipient mice followed by immunization with NP-OVA in alum. The percentage of T_{FH} cells in donor OT-II T cells in the mLN of the recipient mice were analyzed 5 days after immunization. Results represent two independent experiments.



Supplementary Fig. 4. T_H1, T_H2 and T_H17 differentiation of Foxp1-deficient $CD4^+$ T cells. Purified naive $CD4^+$ T cells from tamoxifen-treated *Foxp1*$^{f/f}$Rosa^{YFP} mice (WT) or *Foxp1*$^{f/f}$Cre-ERT2$^+$Rosa^{YFP} mice (cKO) were stimulated by anti-CD3 and anti-CD28 antibodies under T_H1-, T_H2- or T_H17-polarizing culture conditions for 2 days, followed by expansion in T cell medium with IL-2 for two more days. Cytokines were examined by intracellular staining. Results represent three independent experiments.

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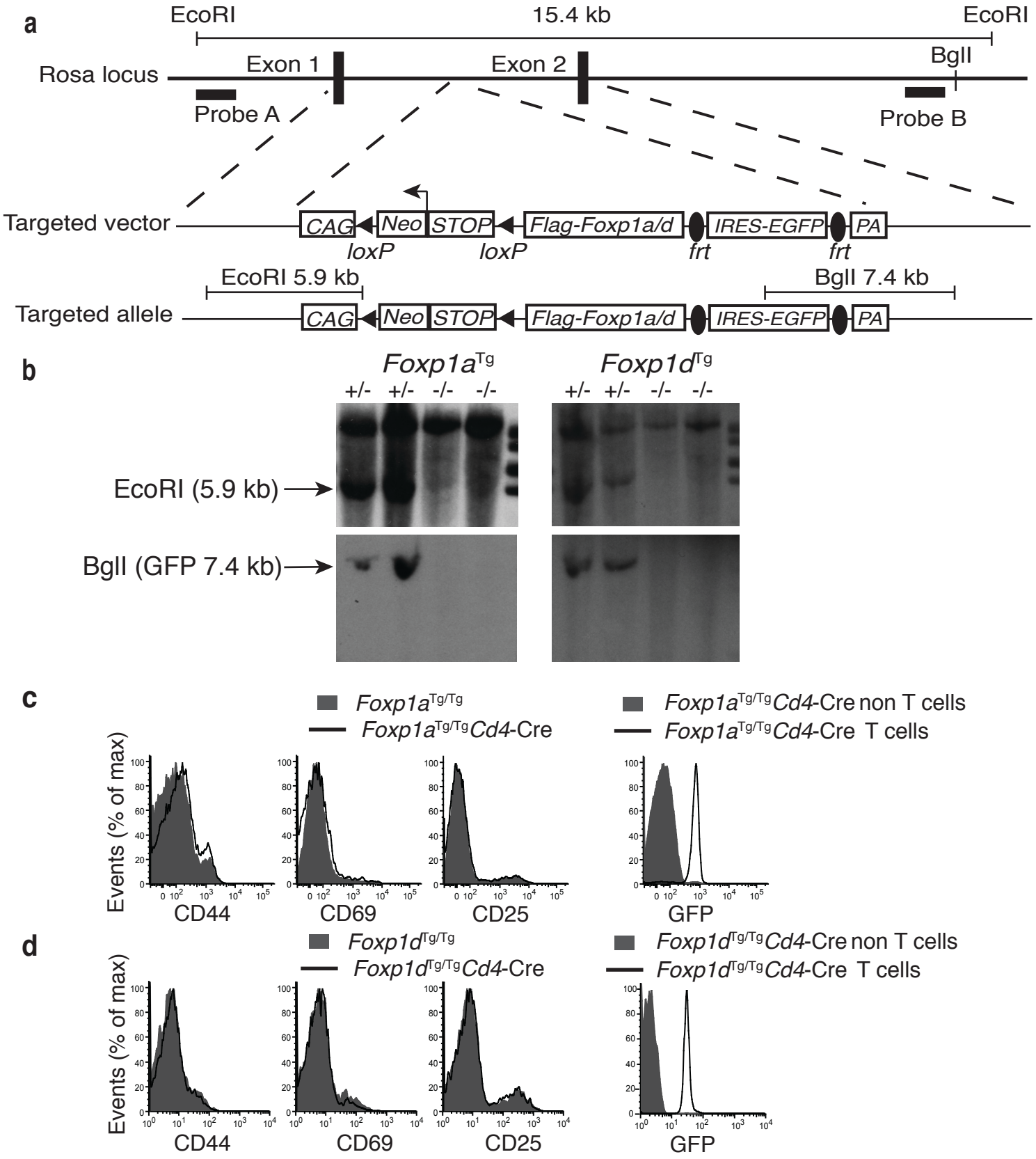
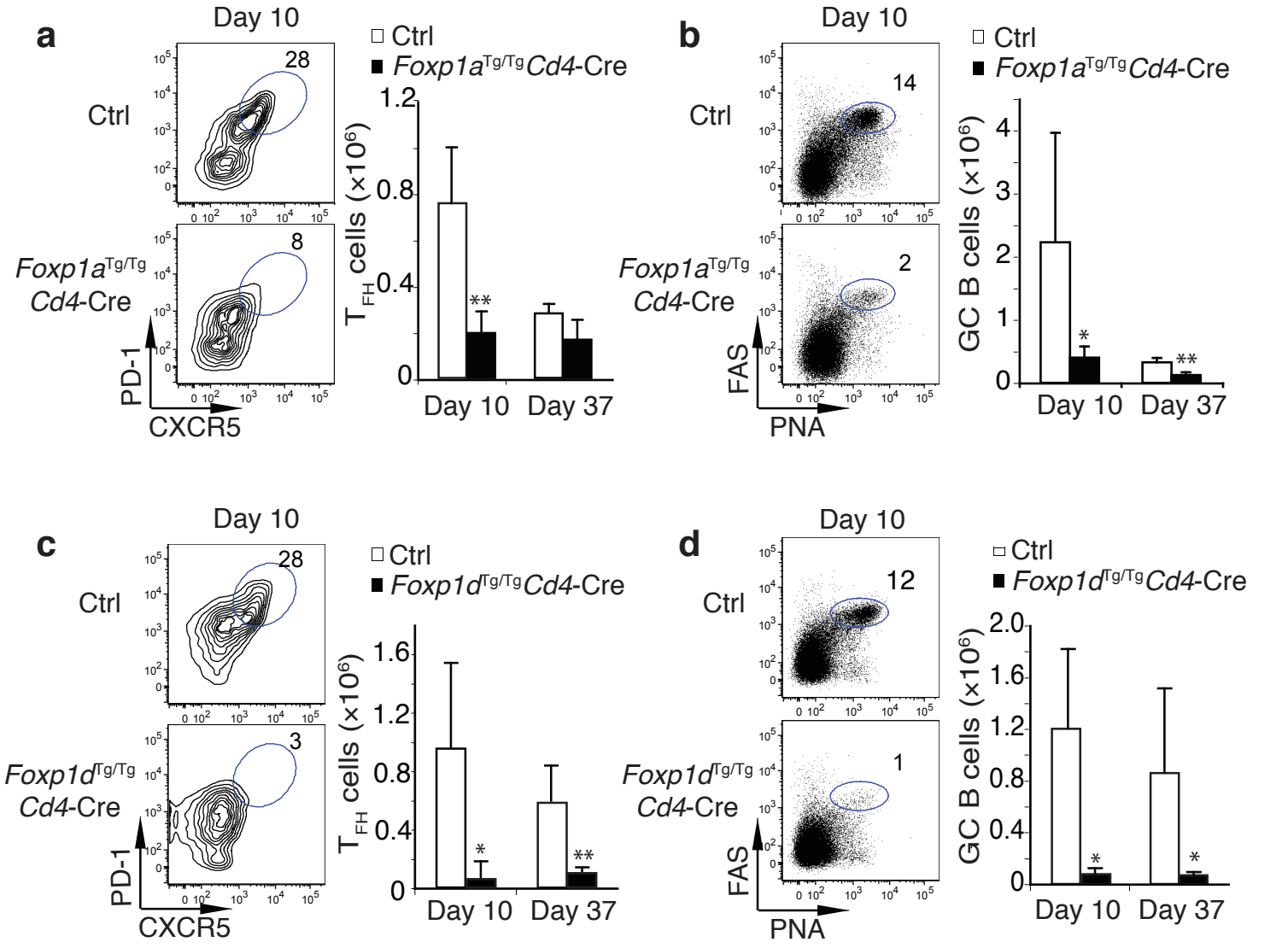
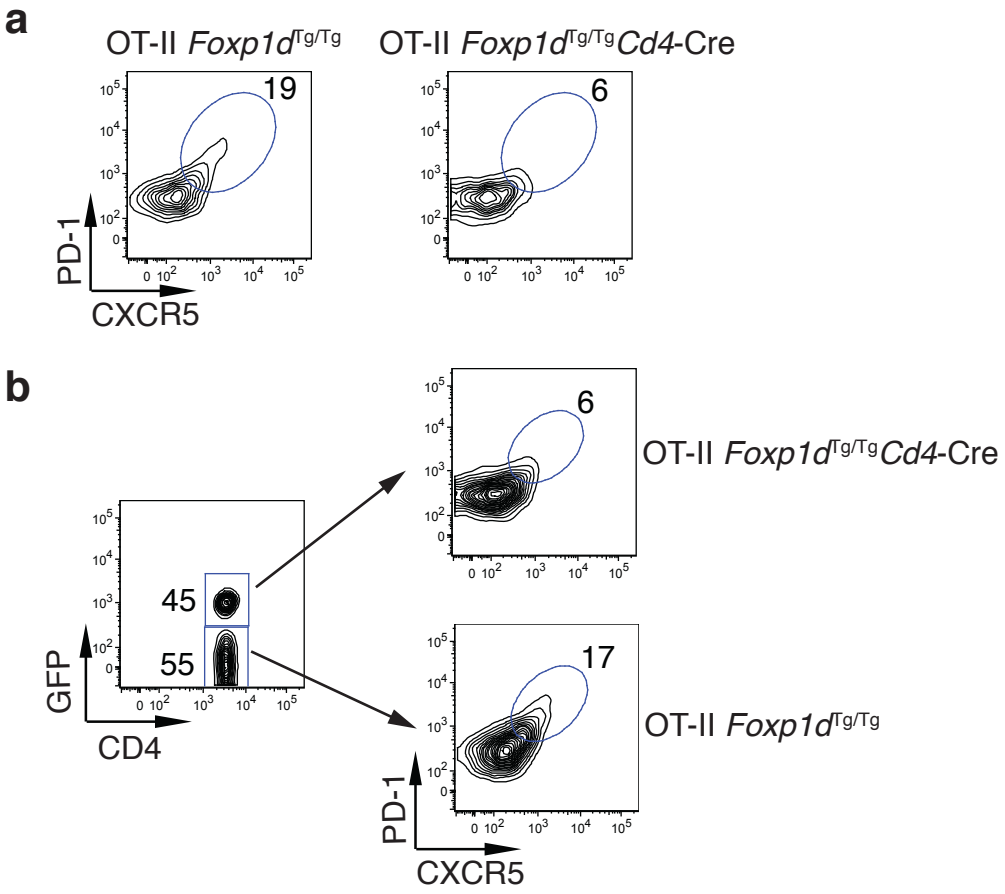


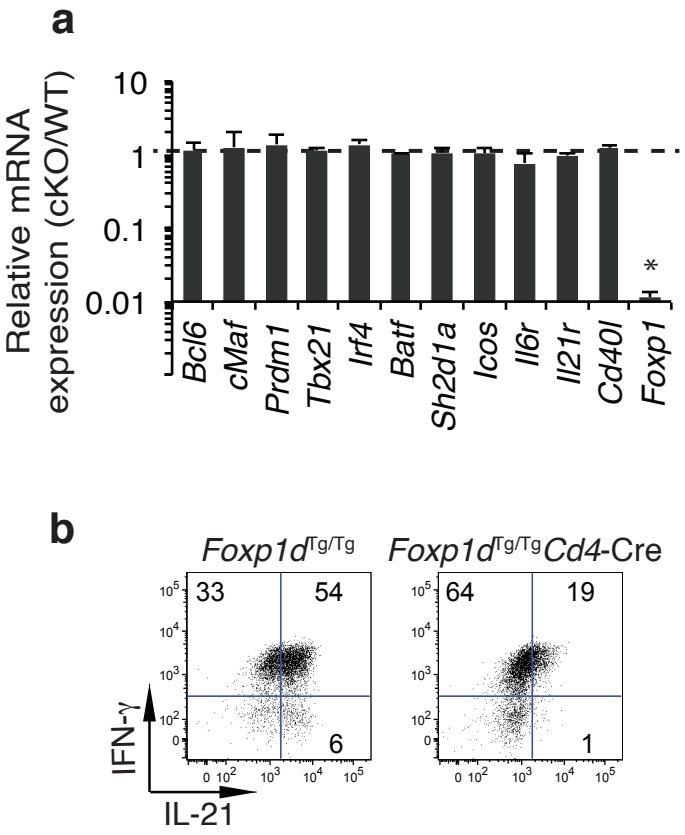
Supplementary Fig. 5. Generation of Foxp1A and Foxp1D conditional transgenic mice. (**a**) Schematic diagram of the targeting strategy for Foxp1A (*Foxp1a*$^{Tg/+}$) or Foxp1D (*Foxp1d*$^{Tg/+}$) conditional transgenic mice. (**b**) Southern blotting analyses of *Foxp1a*$^{Tg/+}$ and *Foxp1d*$^{Tg/+}$ mice. (**c**) Expression of cell surface markers and GFP in *Foxp1a*$^{Tg/Tg}$*Cd4*-Cre CD4$^+$ T cells. (**d**) Expression of cell surface markers and GFP in *Foxp1d*$^{Tg/Tg}$*Cd4*-Cre CD4$^+$ T cells. Results represent at least two independent experiments.



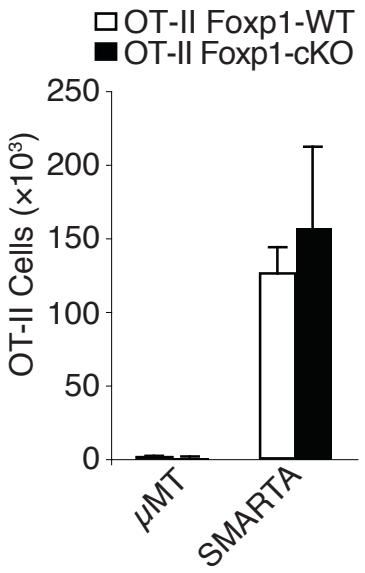
Supplementary Fig. 6. Both Foxp1A and Foxp1D negatively regulate T_{FH} cell development. (**a, b**) *Foxp1a*$^{Tg/Tg}$*Cd4*-Cre mice were infected with influenza virus PR8. The percentages and numbers of PD-1^{hi}CXCR5^{hi} T_{FH} cells (**a**) and GC B cells (**b**) in the spleens of the infected mice were analyzed. *Foxp1a*$^{Tg/Tg}$ mice were used as controls. Error bars represent s.d. (n=5). (**c, d**) *Foxp1d*$^{Tg/Tg}$*Cd4*-Cre mice were infected with PR8 virus. The percentages and numbers of CXCR5^{hi}PD-1^{hi} T_{FH} cells (**c**) and GC B cells (**d**) in the spleens of the infected mice were analyzed. *Foxp1d*$^{Tg/Tg}$ mice were used as controls. Error bars represent s.d. (n=4). Results represent at least two independent experiments. *$P < 0.05$, **$P < 0.01$.



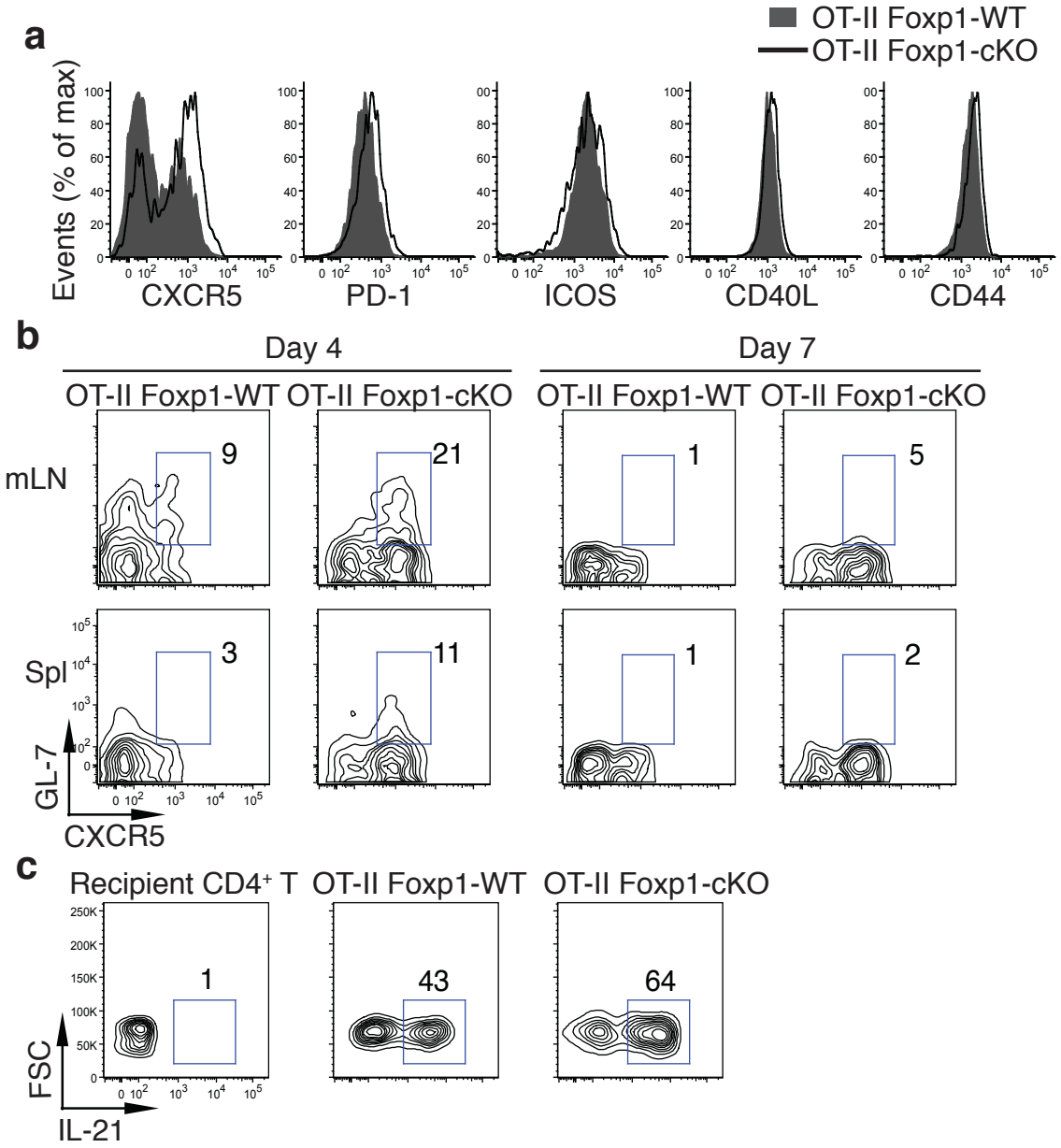
Supplementary Fig. 7. Transgenic expression of Foxp1D suppresses T_{FH} cell differentiation but not the general CD4$^+$ T cell responses. (**a**) Purified naive CD4$^+$ T cells from OT-II *Foxp1d*$^{Tg/Tg}$*Cd4*-Cre or control OT-II *Foxp1d*$^{Tg/Tg}$ mice were transferred into Ly5.1$^+$ C57BL/6 recipient mice followed by immunization with NP-OVA in alum. Seven days after immunization, the percentages of PD-1^{hi}CXCR5^{hi} T_{FH} cells in splenic donor OT-II T cells were analyzed. (**b**) Purified naive CD4$^+$ T cells from OT-II *Foxp1d*$^{Tg/Tg}$*Cd4*-Cre or control OT-II *Foxp1d*$^{Tg/Tg}$ mice were mixed at 1:1 ratio and co-transferred into Ly5.1$^+$ C57BL/6 recipient mice followed by immunization with NP-OVA in alum. The ratios of GFP$^+$ Foxp1D transgenic OT-II T cells to control OT-II and the percentages of T_{FH} cells in splenic donor OT-II T cells were analyzed 7 days after immunization. Results represent two independent experiments.



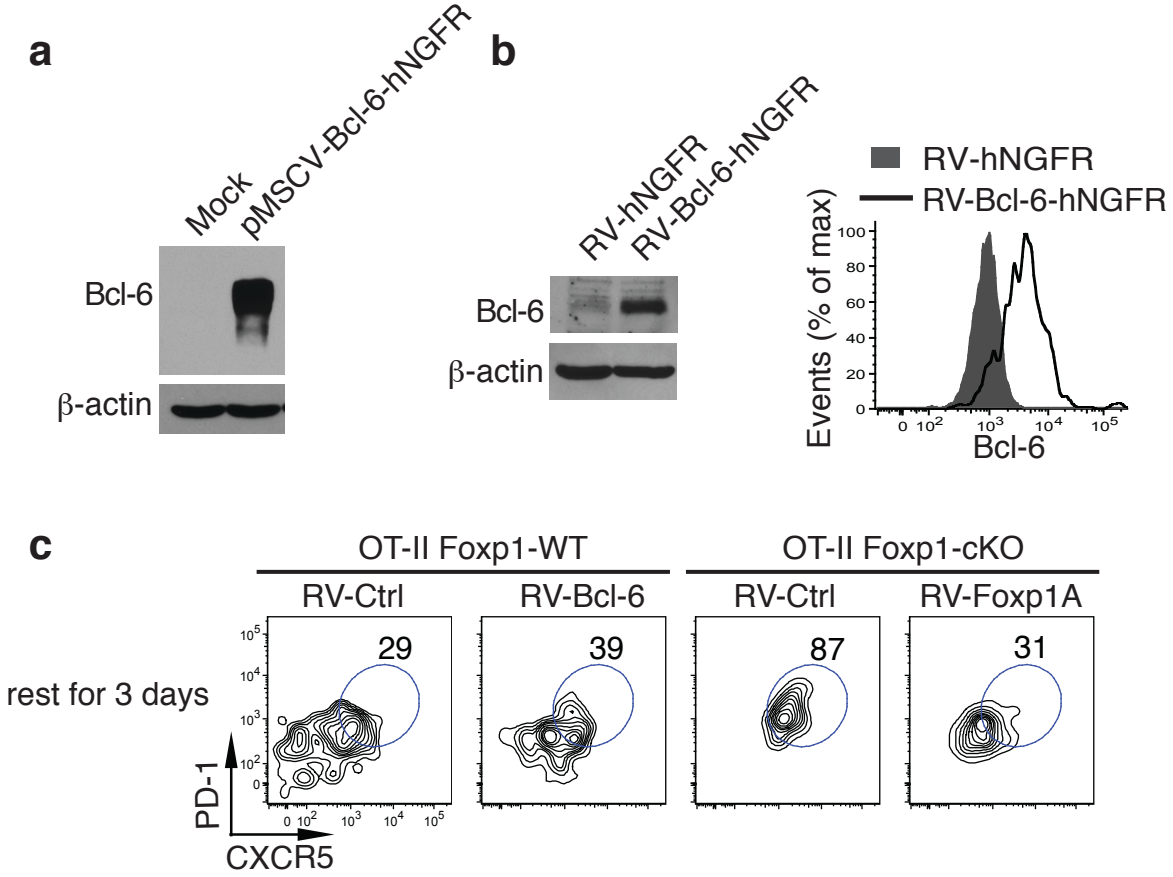
Supplementary Fig. 8. Expression of T_{FH}-related genes in wild-type and Foxp1-deficient T_{FH}-like cells *in vitro* and Foxp1D specifically inhibits IL-21 production *in vitro*.(**a**) Relative mRNA expression in wild-type and Foxp1-deficient T cells activated under T_{FH}-like cell culture conditions *in vitro*. Error bars represent standard error of the mean (s.e.m.). (**b**) Wild-type (*Foxp1d*$^{Tg/Tg}$) or Foxp1D transgenic (*Foxp1d*$^{Tg/Tg}$*Cd4*-Cre) $CD4^+$ T cells were stimulated by anti-CD3 and anti-CD28 antibodies *in vitro* under T_H1-polarizing culture conditions for three days and expanded for additional 2 days in medium. IL-21 production was examined on day 5 by intracellular staining.



Supplementary Fig. 9. Recovery of donor OT-II T cells at the initial stage of the responses in B cell-deficient μMT recipient mice versus B cell-intact recipient mice. The day 3 data of total donor OT-II T cells in the spleens of the recipient mice of **Fig. 7b** and **Fig. 2a** are shown. Error bars represent s.d. (μMT recipients: n=2; SMARTA recipients: n=4). Results represent at least two independent experiments.



Supplementary Fig. 10. T_{FH} cell differentiation of Foxp1-deficient $CD4^+$ T cells in μMT recipient mice. Purified naive OT-II Foxp1-WT and OT-II Foxp1-cKO T cells were transferred into μMT recipient mice followed by immunization with NP-OVA in alum. (**a**) Phenotypes of donor OT-II T cells in the spleens of the recipient mice 5 days after immunization. (**b**) Percentages of $GL7^{hi}CXCR5^{hi}$ GC T_{FH} cells in splenic donor OT-II T cells 4 and 7 days after immunization. (**c**) Percentages of IL-21-producing cells in day 5 splenic donor OT-II T cells by intracellular staining. Results represent at least two independent experiments.



Supplementary Fig. 11. Retroviral over-expression of Bcl-6. (**a**) Immunoblotting of Bcl-6 in HEK293T cells transfected with plasmid MSCV-IRES-hNGFR (Mock) or MSCV-Bcl-6-hNGFR. (**b**) Immunoblotting (left panel) or intracellular staining (right panel) of Bcl-6 in $CD4^+$ T cells infected with RV-Ctrl or RV-Bcl-6 retroviruses. (**c**) Wild-type or Foxp1-deficient naive OT-II T cells transduced with RV-Ctrl, RV-Bcl-6, or RV-Foxp1A were transferred into $Ly5.1^+$ C57BL/6 recipient mice. Three days later, the recipient mice were immunized with NP-OVA in alum. T_{FH} cell differentiation of $hNGFR^+$ cells in the mLN was analyzed 5 days after immunization. Results represent at least two independent experiments.

Supplementary Table 1. Expression and cKO/WT T_{FH} fold change for 118 genes selected as T_{FH} markers based on microarray data (m, shown as blue dots on scatter plot) and additional markers based on literature search (l).

#	IlluminaID	Symbol	m	l	cKO/WT T_{FH}	WT n 1	WT T_{FH} 1	WT T_{FH} 2	cKO n 1	cKO T_{FH} 1	cKO T_{FH} 2
1	ILMN_1230353	*Bcl6*	y	y	1.63	11.1	12.7	13.2	11.8	13.6	13.7
2	ILMN_2725464	*SAP*	y	y	1.12	12.9	13.8	13.7	12.8	14.0	13.9
3	ILMN_2990494	*Cxcr5*	y	y	1.18	11.3	14.6	14.2	11.9	14.6	14.7
4	ILMN_1260060	*Pdcd1*	y	y	1.17	8.4	9.1	8.9	8.5	9.1	9.3
5	ILMN_2435486	*Tnfsf8*	y	y	1.60	10.2	10.8	10.8	10.2	11.6	11.5
6	ILMN_1215795	*Il21*	y	y	2.80	8.5	11.1	11.2	10.4	12.7	12.5
7	ILMN_2931334	*Il4*	y	y	1.33	9.5	12.7	13.5	10.0	13.1	13.9
8	ILMN_2630459	*Cxcr4*	y	y	1.11	10.4	11.1	11.2	10.2	11.0	11.6
9	ILMN_2642800	*Sostdc1*	y		5.98	7.9	9.7	10.0	8.7	12.0	12.8
10	ILMN_2758720	*Sypl*	y		1.02	11.9	12.4	12.6	11.9	12.5	12.6
11	ILMN_2502471	*BC023892*	y		0.82	9.8	8.6	9.1	9.7	8.9	8.1
12	ILMN_3161679	*Serpini1*	y		0.79	8.9	8.2	8.3	8.5	8.0	7.8
13	ILMN_2886610	*Txlnb*	y		1.20	8.1	8.7	8.7	8.3	8.8	9.1
14	ILMN_2718330	*Cish*	y		1.29	11.7	9.9	10.2	12.4	10.6	10.3
15	ILMN_1217180	*Ifitm1*	y		0.67	10.9	9.8	9.5	11.0	9.3	8.8
16	ILMN_1232766	*Sccpdh*	y		1.33	9.5	11.9	11.9	9.4	12.1	12.4
17	ILMN_2707181	*Cd160*	y		1.11	9.9	12.4	12.3	10.2	12.3	12.7
18	ILMN_2645662	*Tmem86a*	y		1.14	8.8	9.5	9.5	8.8	9.5	9.9
19	ILMN_2971286	*Ppp3ca*	y		1.16	11.6	10.5	10.5	11.8	10.9	10.5
20	ILMN_2693403	*Ela1*	y		1.00	9.2	10.5	10.7	9.8	10.4	10.8
21	ILMN_1255860	*Klrd1*	y		1.07	8.8	8.3	8.3	9.2	8.6	8.2
22	ILMN_2902228	*Coro2b*	y		1.15	8.1	10.0	9.8	8.2	9.8	10.4
23	ILMN_2651054	*LOC100047173*	y		0.82	10.5	9.9	9.9	10.8	9.7	9.5
24	ILMN_2589615	*Ptrh1*	y		2.01	9.9	11.4	11.0	10.9	12.3	12.2
25	ILMN_1242024	*Setd4*	y		1.15	10.0	10.7	10.9	9.7	10.9	11.1
26	ILMN_2764727	*Actn2*	y		1.70	10.1	9.0	8.9	11.0	9.6	9.9
27	ILMN_1232537	*Arl4c*	y		0.78	12.6	10.4	11.1	12.2	10.6	10.1
28	ILMN_1221620	*Il18rap*	y		0.89	8.9	8.2	8.0	9.2	7.9	7.9
29	ILMN_2721399	*Ctsw*	y		0.92	10.4	9.2	8.8	11.4	9.1	8.7
30	ILMN_2430813	*Pdlim7*	y		1.01	11.2	12.1	12.1	11.0	11.9	12.3
31	ILMN_1254653	*Angptl2*	y		0.87	8.5	10.5	9.8	8.5	9.9	9.9
32	ILMN_2909782	*Rras2*	y		1.08	10.2	9.6	9.7	10.9	9.9	9.6
33	ILMN_2605819	*Egln3*	y		2.32	10.8	11.7	11.5	11.7	12.8	12.9
34	ILMN_1232901	*Vamp5*	y		1.36	9.0	10.0	9.8	9.4	10.1	10.6
35	ILMN_3159170	*Cd22*	y		0.71	8.3	9.6	9.4	9.0	9.0	9.0
36	ILMN_2637113	*Slc9a9*	y		0.81	10.9	12.0	11.6	10.7	11.5	11.5
37	ILMN_3111877	*Rbms2*	y		1.35	11.4	12.5	12.6	11.3	12.8	13.1

38	ILMN_2445166	*Vdr*	y	1.21	8.5	10.8	10.0	9.1	10.2	11.2
39	ILMN_2642403	*Lmo4*	y	1.78	11.8	13.1	13.5	12.6	13.8	14.5
40	ILMN_2615559	*Dab2ip*	y	1.14	7.9	8.7	9.0	8.1	8.9	9.2
41	ILMN_2631514	*BC017612*	y	0.91	11.3	13.1	12.2	11.6	12.3	12.7
42	ILMN_1224186	*Ica1l*	y	1.58	8.4	9.3	9.5	9.0	10.0	10.1
43	ILMN_2588051	*Tubb2b*	y	1.31	8.5	11.1	10.1	9.1	11.0	11.0
44	ILMN_2788073	*Hmox1*	y	0.38	8.9	11.1	9.9	9.5	9.3	8.9
45	ILMN_2696629	*Itgal*	y	1.15	12.3	13.1	12.9	12.6	13.0	13.4
46	ILMN_1232028	*Itga4*	y	0.67	11.9	11.5	11.4	11.7	11.3	10.4
47	ILMN_2701355	*Nanos1*	y	1.47	9.0	9.6	9.8	9.0	10.1	10.4
48	ILMN_2765759	*Asb2*	y	1.14	11.2	13.5	14.0	11.7	13.6	14.3
49	ILMN_1255220	*Atp9a*	y	0.99	7.9	9.4	8.7	7.9	8.6	9.4
50	ILMN_2754717	*Pear1*	y	0.70	9.5	10.5	10.1	9.1	9.7	9.8
51	ILMN_1225303	*Tox2*	y	0.95	7.7	8.4	8.2	7.9	8.3	8.2
52	ILMN_2743902	*Matk*	y	0.75	10.0	11.5	12.0	9.7	10.9	11.7
53	ILMN_1221920	*Plekhg3*	y	1.25	10.1	11.7	12.4	11.1	12.0	12.8
54	ILMN_2984744	*Emp3*	y	0.51	14.6	14.0	13.9	14.3	13.1	12.9
55	ILMN_2750011	*Galnt10*	y	1.16	12.8	12.0	11.8	12.8	12.3	11.9
56	ILMN_2635232	*LOC100046953*	y	0.83	9.4	8.6	8.7	10.5	8.4	8.4
57	ILMN_2594139	*6330403K07Rik*	y	2.13	9.2	10.2	11.1	10.6	11.3	12.2
58	ILMN_2613923	*Popdc2*	y	0.54	9.1	10.1	10.2	9.4	9.2	9.3
59	ILMN_2789862	*B4galnt4*	y	1.15	8.1	9.7	9.7	9.1	9.7	10.1
60	ILMN_2419138	*Vcl*	y	1.05	10.0	8.2	8.7	9.9	8.7	8.3
61	ILMN_2458765	*Ahnak*	y	0.64	14.4	13.6	13.5	14.5	13.1	12.7
62	ILMN_2744492	*Nhsl1*	y	1.07	7.8	8.4	8.5	7.6	8.4	8.8
63	ILMN_2777598	*Nr2f6*	y	0.79	9.3	10.2	10.1	9.4	9.6	10.1
64	ILMN_2952650	*Klrb1c*	y	0.90	8.6	9.6	10.0	8.7	9.5	9.8
65	ILMN_2988143	*Plac8*	y	0.37	13.6	11.8	11.1	12.6	10.5	9.6
66	ILMN_2795791	*Tmem38b*	y	0.78	11.7	12.2	12.3	11.5	12.0	11.8
67	ILMN_2749448	*Plekho2*	y	0.80	11.7	12.2	12.1	11.8	11.8	11.9
68	ILMN_1253691	*B430201A12Rik*	y	0.89	9.2	8.3	8.6	8.6	8.5	8.1
69	ILMN_2653215	*Nagk*	y	0.78	11.0	11.8	11.6	10.7	11.2	11.4
70	ILMN_1254927	*Ly6c1*	y	0.93	9.8	9.1	8.7	10.0	9.0	8.7
71	ILMN_1246725	*Mrps6*	y	1.12	12.8	13.6	13.6	13.0	13.6	14.0
72	ILMN_2612255	*Lyl1*	y	0.96	7.8	8.4	8.5	8.1	8.6	8.3
73	ILMN_2909597	*Havcr2*	y	0.99	8.3	7.8	7.8	8.5	7.9	7.7
74	ILMN_2813454	*Dnajc15*	y	0.92	13.1	12.4	12.1	13.4	12.1	12.0
75	ILMN_2747196	*Cd82*	y	1.06	13.5	14.3	14.1	14.0	14.1	14.4
76	ILMN_2548010	*Hopx*	y	0.91	9.9	8.8	9.2	9.4	8.9	8.8
77	ILMN_2852904	*Ublcp1*	y	0.74	11.0	12.1	11.7	10.8	11.4	11.5
78	ILMN_2911788	*Tmprss13*	y	0.70	9.7	8.7	8.3	9.0	8.2	7.8
79	ILMN_2713464	*Abhd4*	y	0.75	9.0	10.0	9.8	8.7	9.3	9.6
80	ILMN_2819859	*Tbc1d2b*	y	0.94	12.1	13.0	12.7	12.1	12.6	12.9

81	ILMN_2589662	*Bmp7*	y	0.66	10.9	11.6	11.5	10.3	10.7	11.2
82	ILMN_2909275	*Slc46a1*	y	0.98	7.7	8.5	8.3	7.8	8.2	8.6
83	ILMN_3143621	*2610020H08Rik*	y	1.04	10.0	8.8	9.1	9.7	8.8	9.1
84	ILMN_3026198	*Rnaset2b*	y	0.95	15.0	15.8	15.6	14.7	15.4	15.8
85	ILMN_2765513	*Kif3a*	y	0.94	8.8	9.6	9.4	9.1	9.5	9.4
86	ILMN_1235374	*Decr1*	y	1.02	9.0	9.8	9.9	9.3	9.7	10.0
87	ILMN_2773900	*Glipr2*	y	0.89	13.3	12.3	12.2	13.7	12.1	12.1
88	ILMN_2789077	*Itgb1*	y	1.54	10.2	8.9	9.2	10.8	10.0	9.4
89	ILMN_1228822	*Tsga14*	y	0.91	10.8	11.5	11.4	10.7	11.3	11.4
90	ILMN_1248650	*2900027G03Rik*	y	1.02	8.5	9.2	9.0	8.4	9.0	9.3
91	ILMN_1218504	*Chdh*	y	0.87	9.0	9.7	9.9	8.6	9.4	9.8
92	ILMN_2843394	*Ptgir*	y	0.65	9.7	9.2	9.0	9.5	8.4	8.6
93	ILMN_2733073	*Ryr1*	y	1.13	9.1	10.3	10.3	9.7	10.4	10.6
94	ILMN_2706819	*Rasl11b*	y	0.80	10.2	8.9	8.8	9.4	8.5	8.5
95	ILMN_2634859	*Nek4*	y	1.01	8.1	8.6	8.5	8.2	8.4	8.7
96	ILMN_1239210	*Scn11a*	y	7.86	7.4	8.1	8.4	9.2	10.9	11.6
97	ILMN_1235657	*Rnase4*	y	0.87	8.0	9.4	8.7	8.0	8.7	9.0
98	ILMN_1220101	*Ebi2*	y	0.73	13.0	14.0	13.8	12.4	13.5	13.4
99	ILMN_2695181	*Smtn*	y	0.69	11.9	13.5	12.9	11.6	12.4	13.0
100	ILMN_2748941	*Itfg3*	y	0.71	9.6	10.7	10.4	9.7	10.0	10.2
101	ILMN_1223317	*Lgals3*	y	0.88	10.9	10.2	10.2	12.7	10.5	9.5
102	ILMN_2759933	*Gna15*	y	0.76	10.9	10.0	9.6	11.0	9.4	9.5
103	ILMN_3125606	*D12Ertd647e*	y	0.88	13.3	14.3	14.1	13.1	13.8	14.2
104	ILMN_2416764	*Cacna1d*	y	0.67	11.0	12.6	12.5	10.1	11.8	12.1
105	ILMN_2686975	*Fam129b*	y	0.90	9.0	8.4	8.4	10.0	8.3	8.2
106	ILMN_2759762	*Stard10*	y	0.66	9.8	11.0	10.8	9.4	10.1	10.5
107	ILMN_1228557	*Id2*	y	1.55	10.8	9.1	9.6	11.6	10.1	9.9
108	ILMN_2664224	*Ephx1*	y	0.88	12.7	14.2	14.3	11.8	13.8	14.3
109	ILMN_1217043	*LOC100047762*	y	1.24	9.2	8.6	8.6	9.1	9.0	8.7
110	ILMN_2974064	*Osbpl3*	y	1.36	10.2	11.0	10.9	10.8	11.2	11.6
111	ILMN_2699531	*Rgs10*	y	0.97	14.7	15.3	15.3	14.2	15.3	15.2
112	ILMN_1214364	*E430004N04Rik*	y	1.09	11.9	11.3	11.3	11.6	11.7	11.2
113	ILMN_1254409	*Atxn1*	y	1.22	11.7	9.9	10.4	11.1	11.0	9.9
114	ILMN_2737940	*Grtp1*	y	1.04	7.6	8.3	8.4	7.6	8.3	8.4
115	ILMN_2760963	*Dock6*	y	0.74	10.4	11.4	11.5	9.8	11.1	10.9
116	ILMN_1234698	*Tspan2*	y	0.75	9.4	8.6	8.6	8.7	8.2	8.1
117	ILMN_2767615	*Atp1b1*	y	1.06	9.2	10.2	9.8	9.3	9.9	10.2
118	ILMN_2677824	*Susd4*	y	0.93	7.7	8.4	8.6	7.7	8.2	8.6
119	ILMN_3116935	*Btla*	y	1.14	13.5	13.7	14.0	13.7	14.0	14.0
120	ILMN_2655721	*Stat1*	y	1.36	13.6	13.3	13.3	13.2	13.9	13.6
121	ILMN_2906430	*Icos*	y	1.26	13.5	13.4	13.3	13.7	13.7	13.7
122	ILMN_2608184	*Il6st*	y	0.90	11.6	12.3	12.3	11.0	12.0	12.4
123	ILMN_1248843	*Gata3*	y	1.91	12.4	10.2	12.1	12.2	12.0	12.2

124	ILMN_2446559	*Tnfrsf4*	y	0.99	11.7	11.4	11.3	12.2	11.4	11.4
125	ILMN_1214255	*Cd200*	y	0.75	11.4	11.8	11.7	10.6	11.6	11.1
126	ILMN_2740678	*Slamf6*	y	0.83	10.5	11.6	11.5	10.5	10.9	11.6
127	ILMN_2623699	*Irf4*	y	1.10	11.9	11.1	10.9	12.3	11.1	11.1
128	ILMN_1243741	*Rora*	y	0.95	11.5	10.6	11.3	11.9	10.9	10.7
129	ILMN_2976191	*Stat5a*	y	0.84	10.7	11.4	10.6	11.0	10.8	10.8
130	ILMN_1237448	*Maf*	y	0.85	9.2	10.9	10.0	9.6	10.2	10.2
131	ILMN_1257241	*Cd40lg*	y	1.29	8.5	8.3	8.7	8.5	8.9	8.8
132	ILMN_2981783	*Ptgds2*	y	1.35	8.0	8.1	8.5	8.3	8.7	8.9
133	ILMN_2762983	*Tbx21*	y	1.04	9.2	8.7	8.1	9.9	8.5	8.4
134	ILMN_2667994	*Dnase1l3*	y	0.97	7.9	8.4	8.3	8.4	8.2	8.3
135	ILMN_1259867	*Il21r*	y	0.93	8.1	8.3	8.2	8.3	8.1	8.2
136	ILMN_2760272	*Rorc*	y	0.96	8.3	8.1	8.2	9.1	8.3	7.9
137	ILMN_2791459	*Ifng*	y	1.00	7.8	8.0	7.9	8.8	8.0	7.9
138	ILMN_2741201	*Il17f*	y	1.12	7.7	7.5	7.6	8.1	7.8	7.6
139	ILMN_1233474	*Il2ra*	y	0.96	8.0	7.6	7.8	8.4	7.7	7.6
140	ILMN_2919900	*Ascl2*	y	1.01	8.0	8.0	8.0	7.9	7.8	8.2
141	ILMN_2761900	*Batf*	y	0.73	11.7	12.6	11.3	11.8	11.7	11.4
142	ILMN_2687169	*Id3*	y	0.91	10.9	11.5	11.2	10.4	11.2	11.2
143	ILMN_1255743	*Il6ra*	y	0.98	12.3	12.5	12.3	12.1	12.5	12.3
144	ILMN_2744650	*Prdm1*	y	1.00	7.7	7.5	7.7	7.8	7.8	7.4
145	ILMN_2789321	*Rc3h1*	y	0.94	9.2	9.1	9.0	9.0	9.1	8.8

Supplementary Table 2. Foxp1 deletion leads to increased ICOS expression and Foxo1 activation at early stages of T cell activation.

a. ICOS MFI (mean fluorescence intensity) in **Fig. 6a**.

	Foxp1-WT	Foxp1-cKO	*P* Value*
Day 1	3847	8485	
	3718	7473	
	5529	10978	
Day 2	7366	12671	
	9942	15436	
	9339	14598	0.002

* Paired student's *t*-Test of combined day 1 and day 2 data between Foxp1-WT and Foxp1-cKO.

b. ICOS MFI in **Fig. 6b**.

	OT-II Foxp1-WT	OT-II Foxp1-CKO	*P* Value*
Day 2	1356	2977	
	928	2000	
	1239	2197	
Day 3	2519	2908	
	1874	2818	
	1802	2480	0.007

* Paired student's *t*-Test of combined day 2 and day 3 data between OT-II Foxp1-WT and OT-II Foxp1-cKO.

c. Foxo1 MFI in **Fig. 6c.**

OT-II Foxp1-WT	OT-II Foxp1-CKO	*P* Value*
11128	8290	
18021	14734	
17130	13199	0.009

* Paired student's *t*-Test.