Figure	Panel	Sample size
Fig. 1	Fig. 1d	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=7; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7. Week 1, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 2, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=12; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=3; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Fig. 1e	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. Week 1, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 2, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=5; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=11; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
	Fig. 1f	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=8; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=6. Week 1, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=7; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=2; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=3. Week 2, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=4; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
	Fig. 1g	*Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=7; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
	Fig. 1i	*Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=4; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=2; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=4.
Fig. 2	Fig. 2b	Week 0, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=3. Week 1, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=5; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=4. Week 2, *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=3. Week 5, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=5; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=5.
	Fig. 2c	Week 1, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=4; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=4. Week 5, *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=5.
	Fig. 2d	Week 0, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=4. Week 5, *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=5.
	Fig. 2e	Week 0, *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=4. Week 5, *Foxp3*$^{DTR\text{-}GFP/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR\text{-}GFP/LSL}$*Cd4*^{creERT2}, n=5.
Fig. 3	Fig. 3a	Week 1, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=6. Week 2, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=11; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=3; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.

	Fig. 3f	*Foxp3*$^{LSL/WT}$*Cd4*^{creERT2} GFP+, n=3; *Foxp3*$^{LSL/WT}$*Cd4*^{creERT2} GFP-, n=3; *Foxp3*$^{DTR/WT}$*Cd4*^{creERT2} GFP+, n=3; *Foxp3*$^{DTR/WT}$*Cd4*^{creERT2} GFP-, n=3.
	Fig. 3h	Week 1, *Foxp3*$^{DTR/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR/LSL}$*Cd4*^{creERT2}, n=4. Week 2, *Foxp3*$^{DTR/WT}$*Cd4*^{creERT2}, n=3; *Foxp3*$^{DTR/LSL}$*Cd4*^{creERT2}, n=4. Week 5, *Foxp3*$^{DTR/WT}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{DTR/LSL}$*Cd4*^{creERT2}, n=9.
	Fig. 3j	*Foxp3*$^{DTR/WT}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{DTR/LSL}$*Cd4*^{creERT2}, n=4.
Fig. 4	Fig. 4b	*Foxp3*$^{DTR/Y}$*Cd4*$^{creER/+}$, n=5; *Foxp3*$^{LSL/Y}$*Cd4*$^{creER/+}$, n=5.
	Fig. 4c	Treg percentage, *Foxp3*$^{DTR/Y}$*Cd4*$^{creER/+}$, n=9; *Foxp3*$^{LSL/Y}$*Cd4*$^{creER/+}$, n=7. Treg cell number, *Foxp3*$^{DTR/Y}$*Cd4*$^{creER/+}$, n=5; *Foxp3*$^{LSL/Y}$*Cd4*$^{creER/+}$, n=5.
	Fig. 4d	*Foxp3*$^{DTR/Y}$*Cd4*^{creER} Tconv, n=6; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=7; *Foxp3*$^{DTR/Y}$*Cd4*^{creER} Treg, n=4; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2} Treg, n=7; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Wannabe, n=7.
	Fig. 4f	*Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=9; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=7.
	Fig. 4g	*Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=9; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=7.
	Fig. 4h	*Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=5; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=5.
Fig. 5	Fig. 5c	7 Days, *Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=9; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=9. 1 Mon, *Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=4; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=9. 4 Mon, *Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=4; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=5. 7 Mon, *Foxp3*$^{DTR/Y}$*Cd4*^{creER}, n=4; *Foxp3*$^{LSL/Y}$*Cd4*^{creER} Tconv, n=5.
	Fig. 5j	*Foxp3*$^{LSL/Y}$, 3390 cells; *Foxp3*$^{DTR/Y}$ tdTomato^{-}, 2910 cells; *Foxp3*$^{DTR/Y}$ tdTomato^{+}, 724 cells.
Fig. 6	Fig. 6f	*Foxp3*$^{DTR/Y}$*ROSA*^{Tom}*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/Y}$*ROSA*^{Tom}*Cd4*^{creERT2}, n=2.
	Fig. 6g	γREG^{+} Treg percentage, n=5. γREG^{+} Treg cell number, n=5.
	Fig. 6h	*Foxp3*$^{DTR\text{-}GFP}$*Cd4*^{creERT2}*R26*^{Tom}, adult, n=5; perinatal, n=5. *Foxp3*^{creERT2}*R26*^{Tom}, adult, n=3; perinatal, n=6.
Extended Data Fig. 1	Extended Data Fig. 1f	n=5.
	Extended Data Fig. 1g	No Treg, n=3; Thy1.1^{+} (*Foxp3*$^{LSL/Y}$*Cd4*$^{creERT2/+}$), n=4; Thy1.1^{+} (*Foxp3*$^{LSL/Y}$*Cd4*$^{+/+}$), n=4; Treg (Foxp3^{GFP}), n=4.
Extended Data Fig. 2	Extended Data Fig. 2a	n=6.
	Extended Data Fig. 2d	Week 0, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=7; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=7. Week 1, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=8. Week 2, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=12; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=3; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 2e	Week 0, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=7; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=7. Week 1, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=8. Week 2, *Foxp3*$^{LSL/Y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/Y}$*Cd4*^{creERT2}, n=12; *Foxp3*$^{LSL/Y}$*Cd4*^{creERT2}, n=8.

		Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=3; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
	Extended Data Fig. 2f	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. Week 1, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 2, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=5; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=11; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
	Extended Data Fig. 2g	*Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=7; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=7.
Extended Data Fig. 3	Extended Data Fig. 3b	*Treg number, Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2} control, n=8; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2} FTY720, n=12; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2} control, n=9; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2} FTY720, n=5. tdTomato+ percentage, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2} control, n=2; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2} FTY720, n=10; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2} control, n=9; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2} FTY720, n=5.
	Extended Data Fig. 3c	*Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=12; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 3d	*Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=6; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=12; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
Extended Data Fig. 4	Extended Data Fig. 4d	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=8; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=8; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=3; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=4.
	Extended Data Fig. 4e	Week 0, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=11; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=10; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=8. Week 4, *Foxp3*$^{LSL/y}$*Cd4*^{wt}, n=4; *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=6; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
Extended Data Fig. 5	Extended Data Fig. 5c	Spleen, *Foxp3*^{WT} *Treg*, n=8; *Foxp3*^{LSL} Treg, n=9; Wannabe, n=9. pLN, *Foxp3*^{WT} *Treg*, n=8; *Foxp3*^{LSL} Treg, n=9; Wannabe, n=9. mLN, *Foxp3*^{WT} *Treg*, n=7; *Foxp3*^{LSL} Treg, n=9; Wannabe, n=9. Lung, *Foxp3*^{WT} *Treg*, n=3; *Foxp3*^{LSL} Treg, n=4; Wannabe, n=9. Liver, *Foxp3*^{WT} *Treg*, n=3; *Foxp3*^{LSL} Treg, n=4; Wannabe, n=4. Colon LP, *Foxp3*^{WT} *Treg*, n=3; *Foxp3*^{LSL} Treg, n=4; Wannabe, n=4.
Extended Data Fig. 7	Extended Data Fig. 7a	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=10; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=10.
	Extended Data Fig. 7c	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=2; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=2.
	Extended Data Fig. 7d	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 7e	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 7f	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.

Extended Data Fig. 8	Extended Data Fig. 8a	Spleen, pLN, lung, liver, colon LP, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. mLN, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 8b	Spleen, pLN, lung, liver, colon LP, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. mLN, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 8c	Spleen, pLN, lung, liver, colon LP, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5. mLN, *Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=4; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 8d	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
	Extended Data Fig. 8f	1.5 mon, n=2; 3 mon, n=4, 5 mon, n=2; 7 mon, n=5.
	Extended Data Fig. 8g	1.5 mon, n=2; 3 mon, n=4, 5 mon, n=2; 7 mon, n=5.
Extended Data Fig. 9	Extended Data Fig. 9b	*Foxp3*$^{DTR\text{-}GFP/y}$*Cd4*^{creERT2}, n=5; *Foxp3*$^{LSL/y}$*Cd4*^{creERT2}, n=5.
Extended Data Fig. 10	Extended Data Fig. 10c	Spleen, pLN, mLN, n=4.
	Extended Data Fig. 10e	Spleen, pLN, mLN, n=4.
	Extended Data Fig. 10f	Spleen, pLN, mLN, lung, liver, colon LP, thymus, n=5.
	Extended Data Fig. 10g	Perinate, n=4; adult, n=4.