



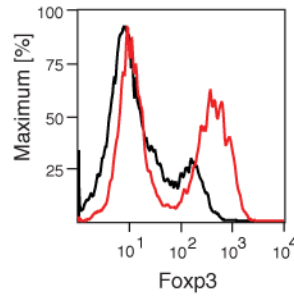
**Table S1. Frequency of Synapse Formation among Interactions of iDCs with Treg and Naive Th Cells, as well as Th::*Nrp-1* and Th::*control* Cells**

	Relative to Interactions [%]				Relative to Cells [%]			
	Treg n=66	Th n=43	Th:: <i>Nrp-1</i> n=38	Th:: <i>control</i> n=38	Treg	Th	Th:: <i>Nrp-1</i>	Th:: <i>control</i>
organized synapses	12	9	21	22	5	2	9	5
close contacts	32	37	37	44	13	7	17	11
loose contacts	56	54	42	34	24	10	19	8
no contact					58	81	55	76

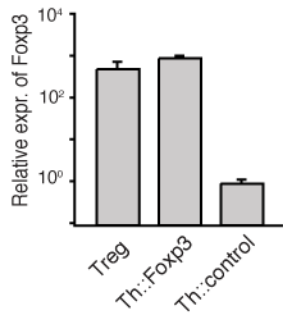
**A**

m6ph[Foxp3]	LTR (MLV)	Foxp3	IRES	HisD	LTR (MLV)
m6p8[Foxp3]	LTR (MLV)	Foxp3	IRES	rCD8a	LTR (MLV)
m6ph[GFP]	LTR (MLV)	GFP	IRES	HisD	LTR (MLV)
m6p8[GFP]	LTR (MLV)	GFP	IRES	rCD8	LTR (MLV)
m6ph[control]	LTR (MLV)	bsd	IRES	HisD	LTR (MLV)
m6p8[control]	LTR (MLV)	bsd	IRES	rCD8	LTR (MLV)

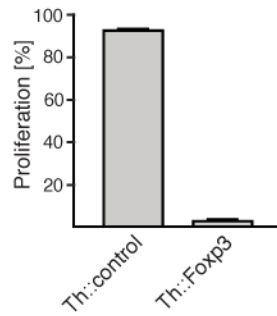
**B**



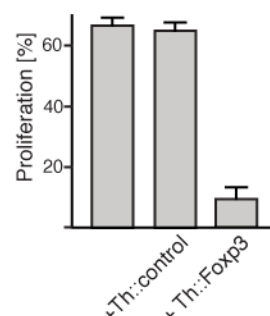
**C**



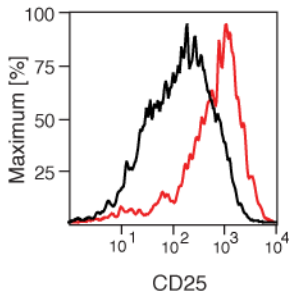
**D**



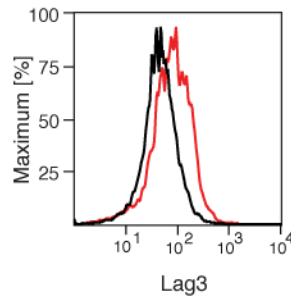
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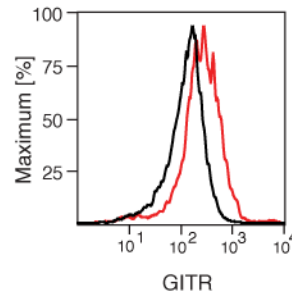
**F**



**G**



**H**



**Figure S1. Foxp3 Transduction of Th Cells**

(A) Foxp3 was amplified from Balb/c cDNA and cloned into the retroviral vectors m6ph and m6p8 carrying an IRES Histidinol-Dehydrogenase (HisD) or IRES GPI-linked ratCD8 cassette. Equivalent constructs in which the Foxp3 was replaced by GFP or blasticidine-S-deaminase (bsd) were used as controls.

(B) FACS analysis of Foxp3 expression in Th::Foxp3 cells (red) and freshly isolated CD4<sup>+</sup> cells (black).

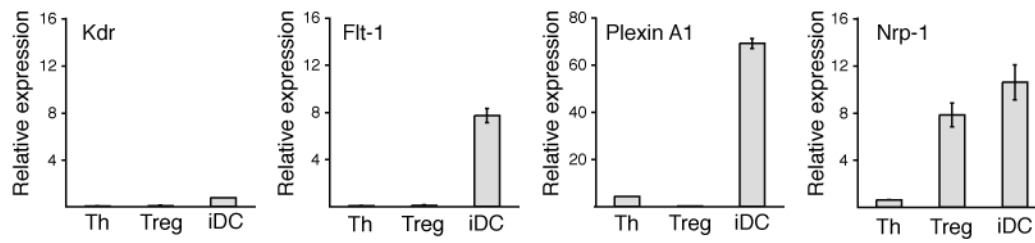
(C) Foxp3 mRNA levels (normalized to Hprt levels) in CD4<sup>+</sup>CD25<sup>+</sup> cells and Th::Foxp3 or Th::control (bsd) cells.

(D) Proliferation of Th::Foxp3 or Th::control (GFP). The bars show the relative numbers of proliferated cells compared to an internal standard (CaliBRITE beads).

(E) Assessment of suppressive activity of Th::Foxp3 or Th::control (bsd). The percentage of cells that have proliferated after 3 days is shown.

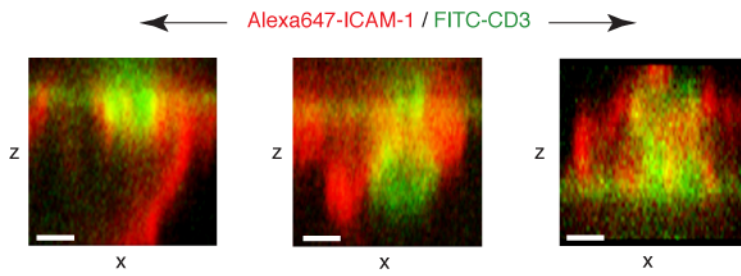
(F-H) Flow-cytometric analysis of Th::Foxp3 cells. (F) CD25, (G) Lag3 and (H) GITR expression in Th::Foxp3 (red) or Th::control (bsd) (black) cells.

All error bars show the standard error of the mean from triplicates.



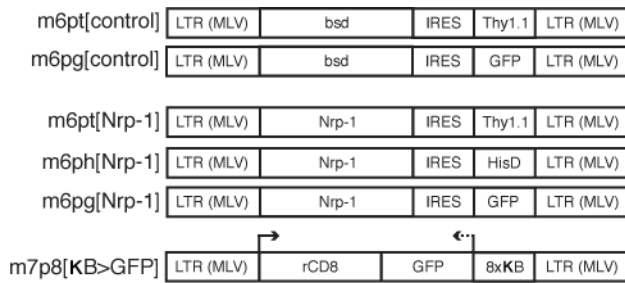
**Figure S2. Comparative Analysis of the Expression of Nrp-1 and Its Coreceptors in Th Cells, Treg Cells, and iDCs**

Relative expression of Kdr (VEGF-R2), Flt-1 (VEGF-R1), Plexin A1 and Nrp-1 in CD4<sup>+</sup>CD25<sup>-</sup> Th cells, CD4<sup>+</sup>CD25<sup>+</sup> Treg cells, and iDCs. mRNA levels were measured by quantitative real-time PCR and normalized to Hprt. All error bars show the standard error of the mean from duplicates.



**Figure S3. Representative Examples of Organized Synapses**

Projection of zx confocal images spanning 0.5  $\mu\text{m}$  in the y direction in the area of the contact zones between DO11.10 x SCID Th cells and ova-loaded [100 $\mu\text{g/ml}$ ; 12 hr] iDCs. White scale bars on the images correspond to 2  $\mu\text{m}$ .



**Figure S4. Retroviral Constructs**

Nrp-1 was amplified from the image clone #6409596 and cloned into the retroviral vectors m6pt, m6ph and m6pg carrying an IRES mouse Thy1.1 marker, an IRES Histidinol-Dehydrogenase (HisD) or IRES GFP cassette. Equivalent constructs in which the Nrp-1 was replaced by GFP (see Figure S1) or blasticidine-S-deaminase (bsd) were used as controls. The m7p8[κB>GFP] is a retroviral vector on which the constitutive expression of a GPI-linked rat CD8 is driven in the ‘forward’ direction and the NF-κB-inducible expression (8 concatenated κB-sites) of GFP in the ‘reverse’ direction