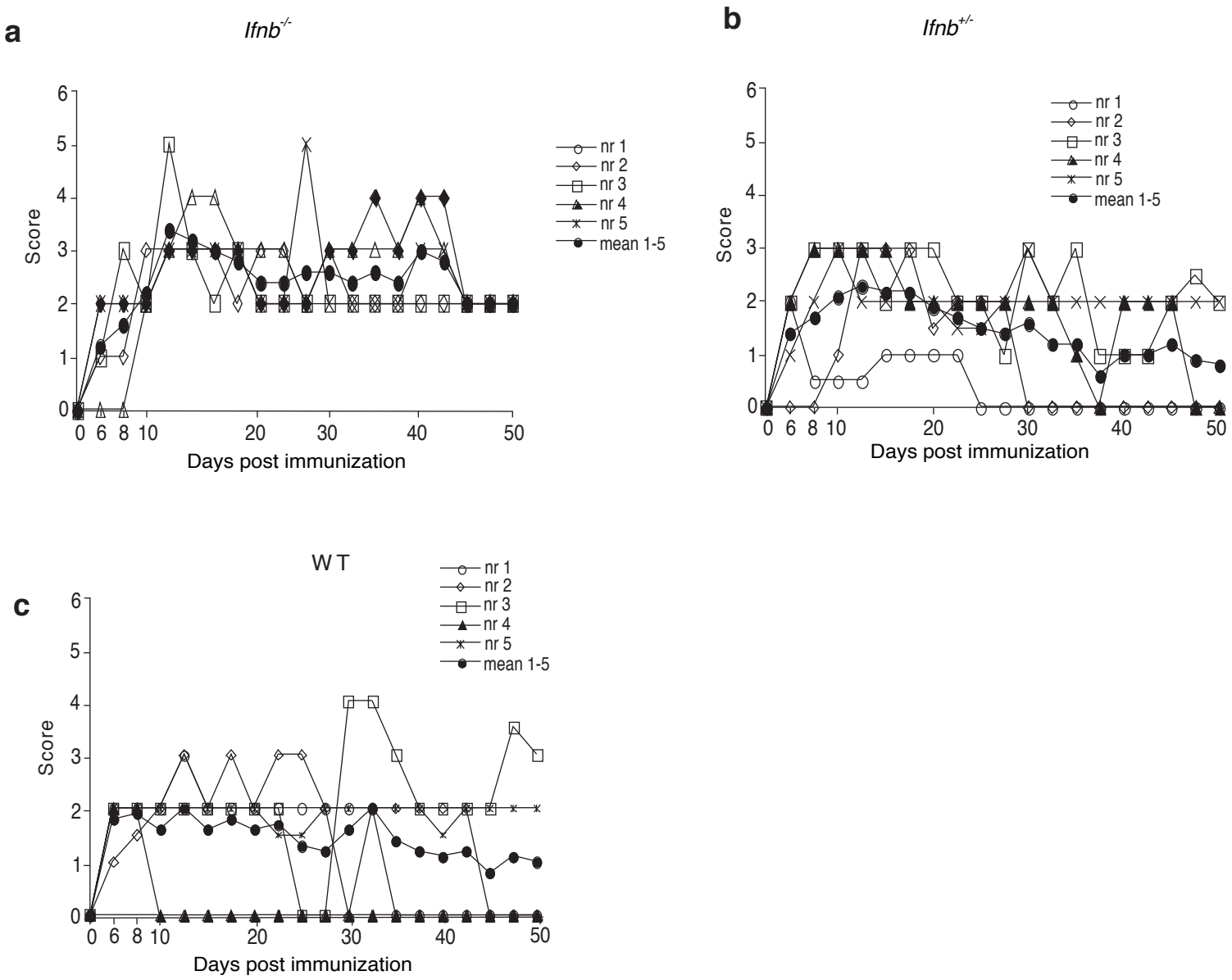
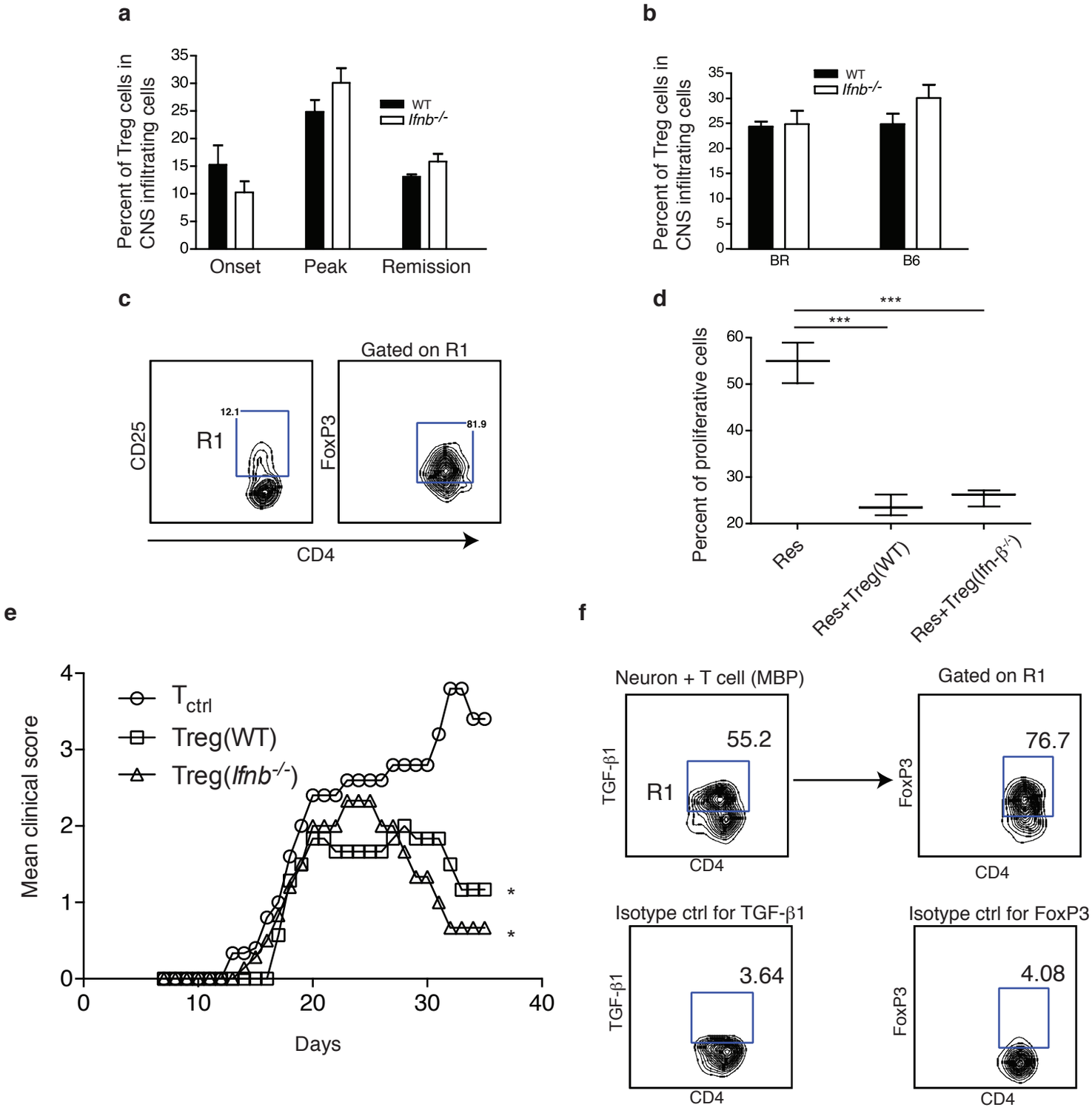
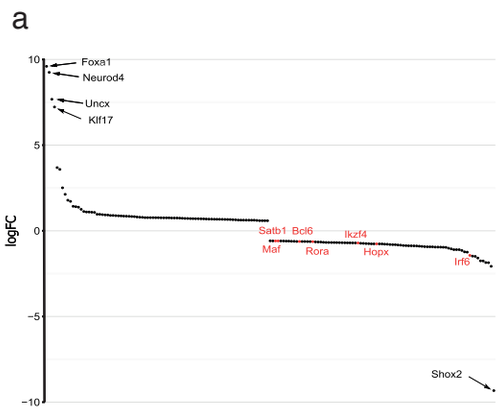


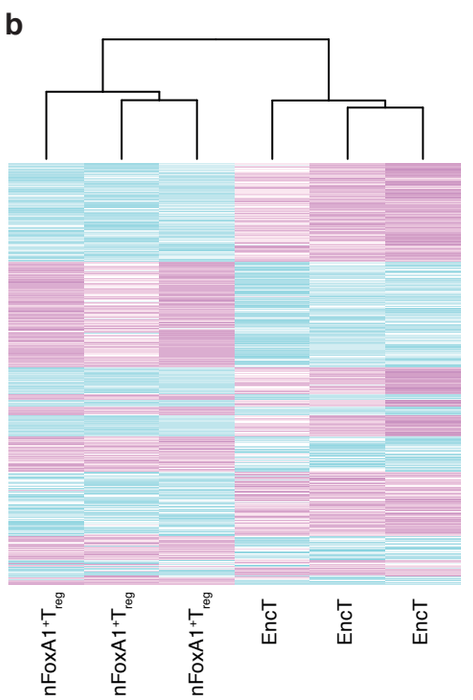
Supplementary Figure 1. *Ifnb*$^{-/-}$ mice develop severe relapsing-remitting form of EAE. (a, b, c) Disease development in five representative EAE-affected mice with different disease courses in *Ifnb*$^{-/-}$, heterozygous (HT/*Ifnb*$^{+/-}$), and WT groups: mice developed different rate number of relapses, with different duration of remission in between. The mean score per group from these five selected individuals with EAE is also shown for the respective groups to demonstrate that due to variability in disease development, the mean score at each time point becomes relatively low, although all five clearly suffer from severe EAE. As no statistical differences were found between WT and HT mice, we will refer to them as WT in the rest of figures. Data are representative of two independent experiments (N = 5).

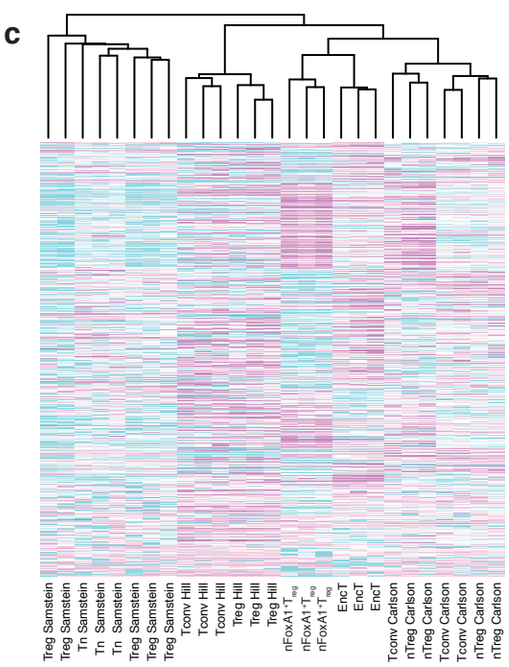


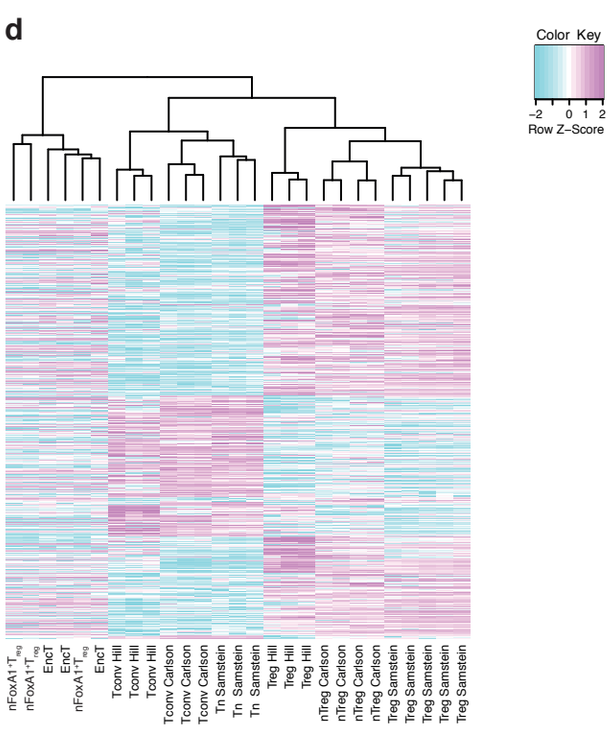
Supplementary Figure 2. Treg cells ($CD4^+CD25^+FoxP3^+$) are not defective in *Ifnb*$^{-/-}$ mice capable of suppressing EAE. (**a**) Percent of $CD4^+FoxP3^+$ Treg cells in the CNS of C57BL/6 WT and *Ifnb*$^{-/-}$ mice after active MOG_{35-55}-induced EAE. *n*=3-5 mice per group. (**b**) Percent of $CD4^+Foxp3^+$ Treg cells in the CNS of C57BL/B10.RIII and C57BL/6 mice during peak of diseases after induction of EAE with MBP_{89-101} and MOG_{35-55} respectively. (**c**) FACS dot plots show gating strategy for $CD4^+CD25^+$ T cells purification, which are also mainly $FoxP3^+$ T cells. (**d**) CFSE-labeled purified murine $CD4^+$ T cells were activated with plate-coated anti-CD3 antibody for 24 hours that served as responder T cells, then co-cultured with purified $CD4^+CD25^+$ T cells from WT and *Ifnb*$^{-/-}$ mice for an additional 24 hours. Bars show the percent of proliferating responder T cells after co-cultures. Data are mean± s.d. *n*=3, ***$P < 0.001$, using one-way ANOVA with Newman-Keuls post hoc test for multiple comparison correction. (**e**) Adoptive EAE was established in irradiated *Ifnb*$^{-/-}$ C57BL/6 mice by transferring 5-10x10^6 splenocytes from MOG_{35-55} immunized mice, day 0 and 13. Purified natural $CD4^+CD25^+$ Treg cells, or TGF-β-induced Treg cells were used for treatment. No functional differences were seen between nTreg cells and iTreg cells hence the results are combined. Groups received either equal number of Ctrl activated T-cells, Treg cells from WT or *Ifnb*$^{-/-}$ mice. Data are the mean of 12-14 mice per group. No differences were found in EAE-suppressive capacity of WT vs. *Ifnb*$^{-/-}$ Treg cells, but both groups equally suppressed EAE, *$P < 0.05$ using one-way ANOVA Kruskal-Wallis test with Dunn's multiple comparisons test for multiple comparison correction. (**f**) FACS dot plots show neuron-induced Treg cells R1-gated ($CD4^+TGF\text{-}\beta1^+$ T cells) are also $FoxP3^+$ Treg cells (upper panels), isotype controls (lower panels).

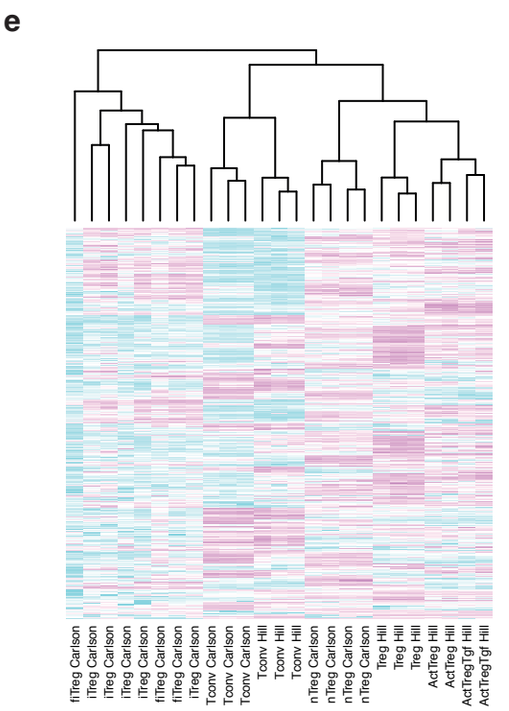


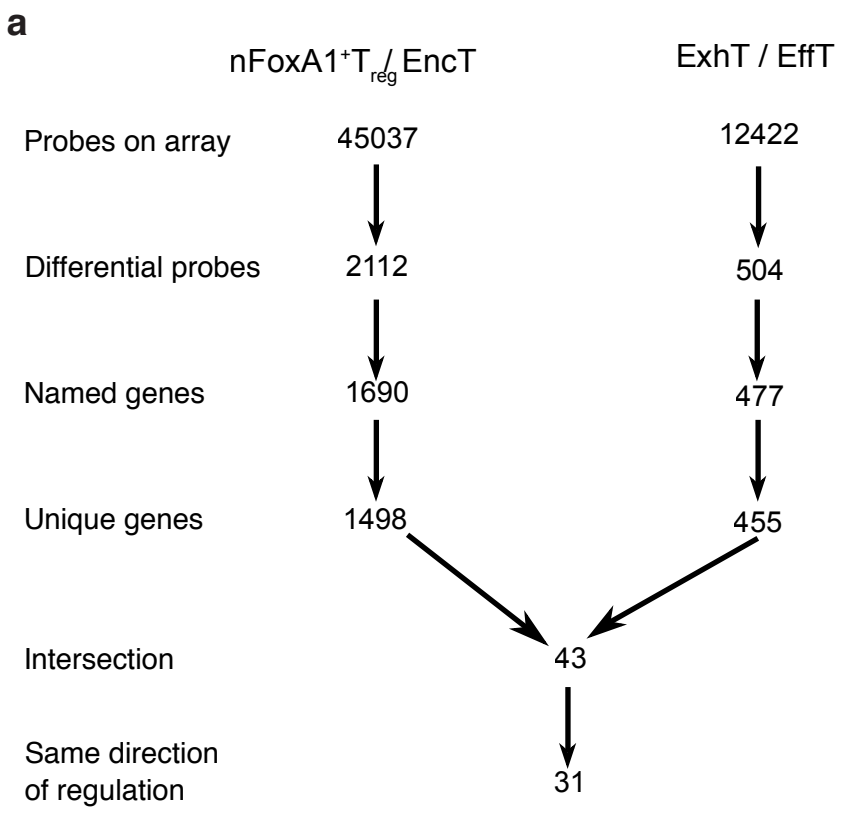
Supplementary Figure 3. FoxA1$^+$ T$_{reg}$ has distinct gene profile than known Tregs. (a) nFoxA1$^+$ T$_{reg}$ Transcription Factor profile. Plot of transcription factor genes differentially regulated in nFoxA1$^+$ T$_{reg}$ cells vs. their progenitor EncT-cells. Y-axis is log2 of fold change. The only relevant transcription factors known to regulate Treg signature (Fu et al. 2012 Nat Imm) were found to be downregulated in nFoxA1$^+$ T$_{reg}$ indicated in red. (b) Heatmap of nFoxA1$^+$ T$_{reg}$ gene profile. Genes on the y-axis correspond to nFoxA1$^+$ T$_{reg}$ gene profile, which is based on differentially regulated genes between nFoxA1$^+$ T$_{reg}$ cells and EncT-cells. (c) Heatmap of nFoxA1$^+$ T$_{reg}$ gene profile in comparison to the published gene expression sets of n/iTreg cells. All included gene sets are normalized together. Genes on the y-axis correspond to nFoxA1$^+$ T$_{reg}$ profile. (d) Heatmap of Treg signature genes (the published gene expression sets) in comparison to nFoxA1$^+$ T$_{reg}$ data sets. All included gene sets are normalized together. Genes on the y-axis correspond to Treg signature (Hill et al. 2007 Imm). (e) Heatmap of nTreg signature genes in comparison to iTreg cells (the published gene expression sets). All included gene sets are normalized together. Genes on the y-axis correspond to Treg signature (Hill et al. 2007 Imm).

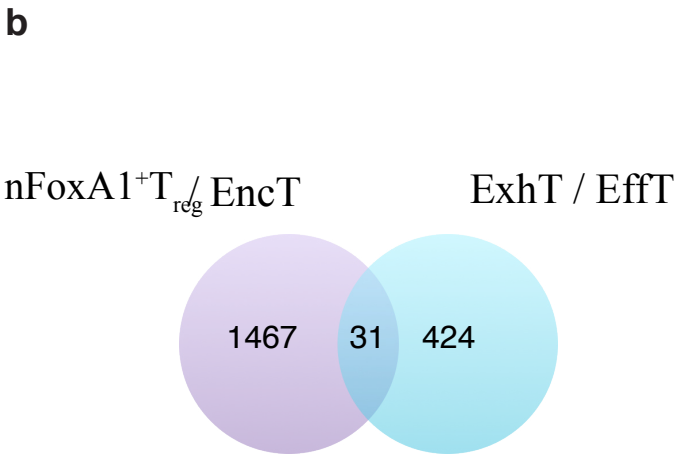










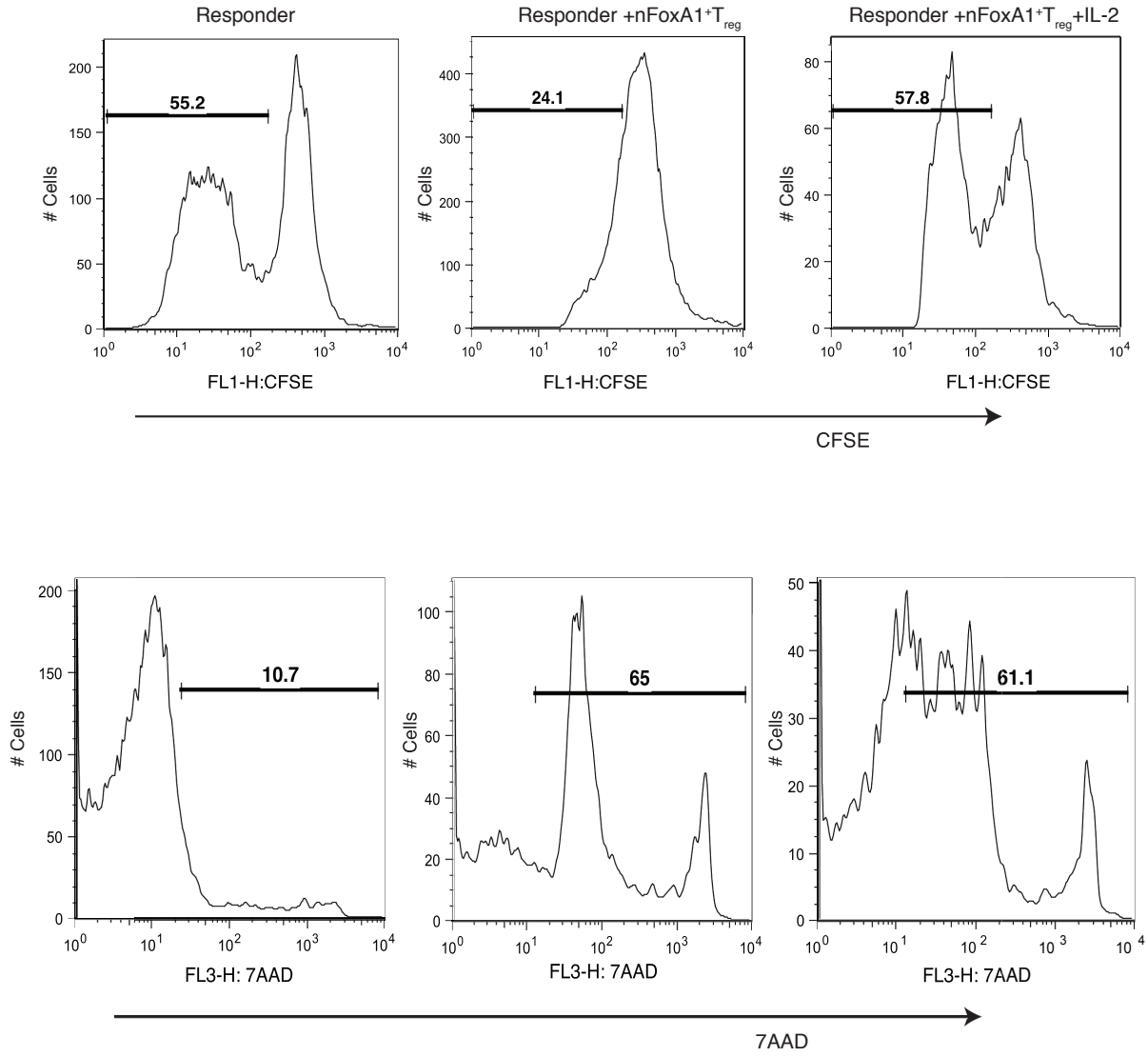


Supplementary Figure 4. nFoxA1^{+}T$_{reg}$ cells have distinct gene signature and surface markers compared to Exhausted T cells. (a) Work flow used to analyze gene expression profiles of the available data-set for exhausted T cells (ExhT-cells) (Wherry et. al, Immunity 2007, 27, 670–84) to compare with nFoxA1^{+}T$_{reg}$ cells . (b) Venn diagram of genes differentially regulated in nFoxA1^{+}T$_{reg}$ cells and ExhT cells. nFoxA1^{+}T$_{reg}$ cells "signature" consists of 1498 genes (2112 probe sets, non-assigned probes and probes matching to multiple genes included), and Exh-CD8+ T cell "signature" consists of 455 genes (504 probes). These two sets share only 43 genes, 31 of which are regulated in the same direction (i.e. up or downregulated in both exhausted and nFoxA1^{+}T$_{reg}$ cells). After adjusting for the probe sets that were not included in the ExhCD8+ T microarray, 658 genes remained in nFoxA1^{+}T$_{reg}$cells "signature", and 407 in ExhCD8+ T cell "signature", and the number of overlap genes (31) remained the same. Additional analyses were performed using analgous approach to compare nFoxA1^{+}T$_{reg}$ cells with exhausted CD8/CD4 T cells from (Doering et al. Immunity 2012, 37, 1130–1144) and (Quigley et al. Nature medicine 2010, 16, 1147–51) datasets. Overlapping genes were only 6 (data not shown).

Supplementary Table VI. Experimentally confirmed protein expression for nFoxA1^{+}T$_{reg}$(CD4^{hi}Pd-l1^{hi}/EncT) and ExhT-cells (ExhT-cells/effectorT-cells).

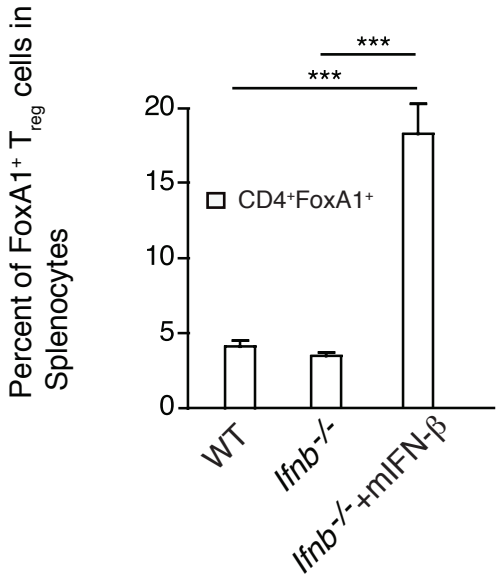
Protein	FoxA1^{+}T$_{reg}$	Exhausted CD8 T	Exhausted CD4 T
Pd-l1	hi	NA	NA
Ctla4	-	+	+
Pd1	+	hi	hi
CD4	hi	-	+
CD8	-	+	-
CD47	+	NA	NA
CD69	+	+	+
FoxA1	+	NA	NA
FoxP3	-	NA	NA
IL10	-	+	NA
TGFb	-	+	+
IFNg	-	-	-
TNFa	-	-	-
IL13	-	NA	-

The confirmed protein expression on reported ExhT-cells are based on the following reports: *Wherry et. al, Immunity 2007, 27, 670–84, Golden-Mason et.al. Journal of virology 2009, 83, 9122–30, Doering et al. Immunity 2012, 37, 1130–1144, Quigley et al. Nature medicine 2010, 16, 1147–51.*



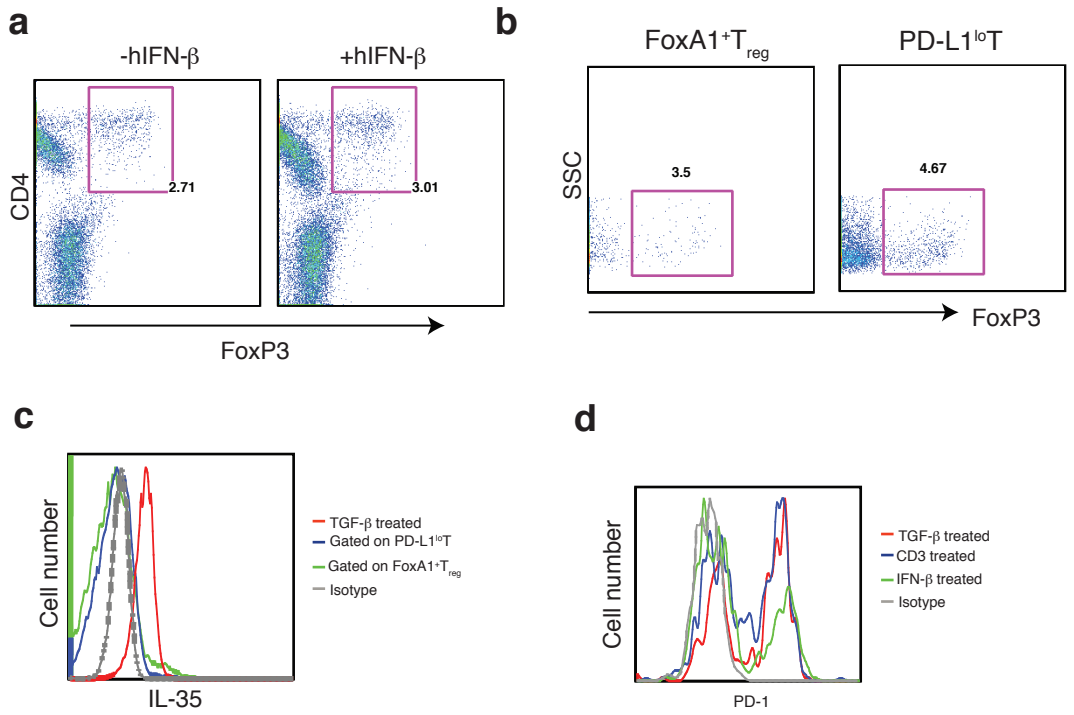
Supplementary Figure 5. Addition of IL-2 has partial effect on inhibitory activity of nFoxA1^{+}T$_{reg}$ cells.

FoxA1^{+}T$_{reg}$ cells were purified on the basis of their surface expression of CD4^{hi}Pd-l1^{hi} by FACSAria from OVA-activated OT-II cells co-cultured with CGNs. Purified CD4+ T cells were labeled with CFSE and activated with anti-CD3/anti-CD28 for 24 hours (responder T cells), and then co-cultured with purified FoxA1^{+}T$_{reg}$ cells for an additional 24 hours. Where indicated recombinant IL-2 (2.4 pg/ml) was added to the co-cultures. FACS histograms show proliferative (CFSE) or dead (7AAD) responder T cells alone and after co-culture with FoxA1^{+}T$_{reg}$ cells (with or without IL-2). IL-2 addition rescues FoxA1^{+}T$_{reg}$ -inhibition of cell proliferation, while there is no effect on cell death of activated responder T cells.



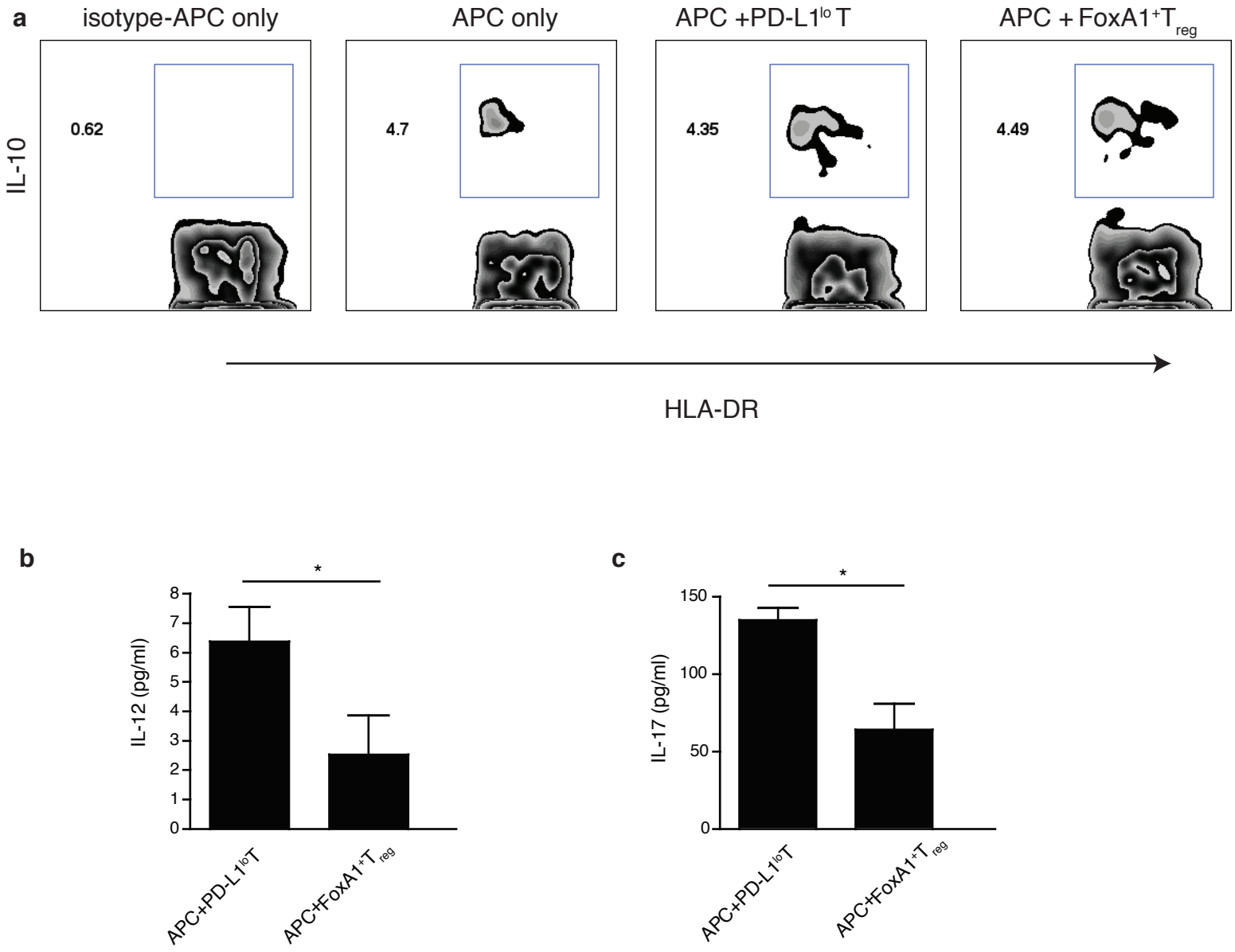
Supplementary Figure 6. IFN-β-treatment of *Ifnb*$^{-/-}$ mice with EAE leads to generation of FoxA1^{+}T$_{reg}$ cells ***in vivo*.**

MOG_{35-55}-EAE was induced in WT and *Ifnb*$^{-/-}$ mice in C57BL/6 background. FoxA1^{+}T$_{reg}$ cells were generated upon treatment of *Ifnb*$^{-/-}$mice with recombinant mIFN-β (5000 U/ml x 3 times). Data indicate successful generation of FoxA1^{+}T$_{reg}$ (CD4^{+}FoxA1^{+} T cells) in the spleens of *Ifnb*$^{-/-}$ mice upon mIFN-β-treatment. Bars are the mean±s.d. of 3-5 mice per group, ***$P < 0.001$ using one-way ANOVA with Newman-Keuls post hoc test for multiple comparison correction.



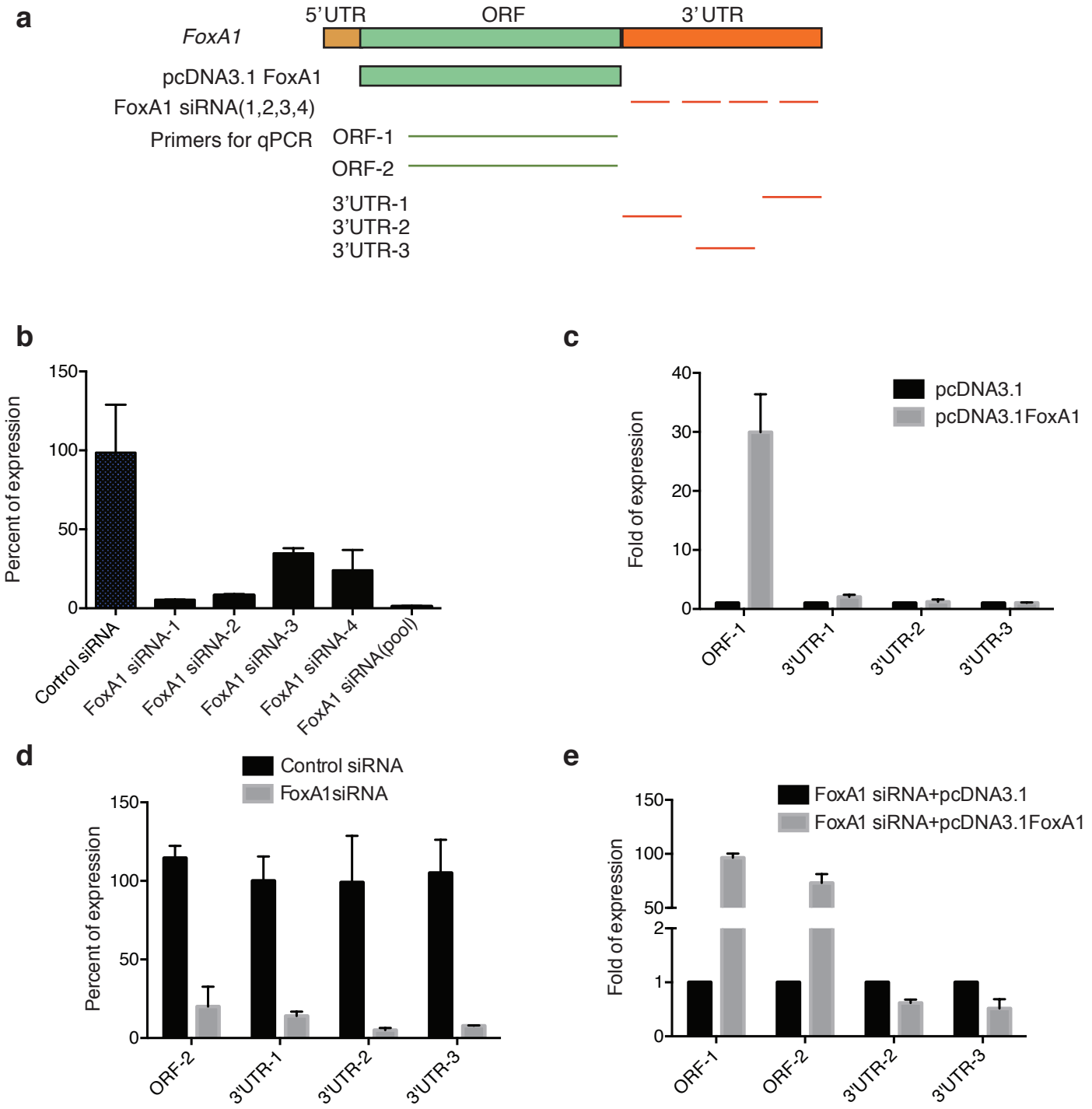
Supplementary Figure 7. hIFN-β treatment do not lead to the generation of Treg cells expressing FoxP3 or IL-35^{+} cells.

Purified CD4^{+} T cells from healthy blood donors were treated *in vitro* with or without recombinant hIFN-β (1000U/ml) for 3 days. **(a)** FACS dot plots show no differences in the FoxP3 expression with or without hIFN-β treatment. **(b)** FoxP3 expression do not differ in purified iFoxA1^{+}T$_{reg}$ cells (R1-gated) compared to PD-L1^{lo}T cells (R2-gated). **(c)** Histograms of IL-35 expression are shown in gated iFoxA1^{+}T$_{reg}$ cells in comparison with PD-L1^{lo}T cells. **(d)** FACS histogram shows PD-1 expression after different treatments.

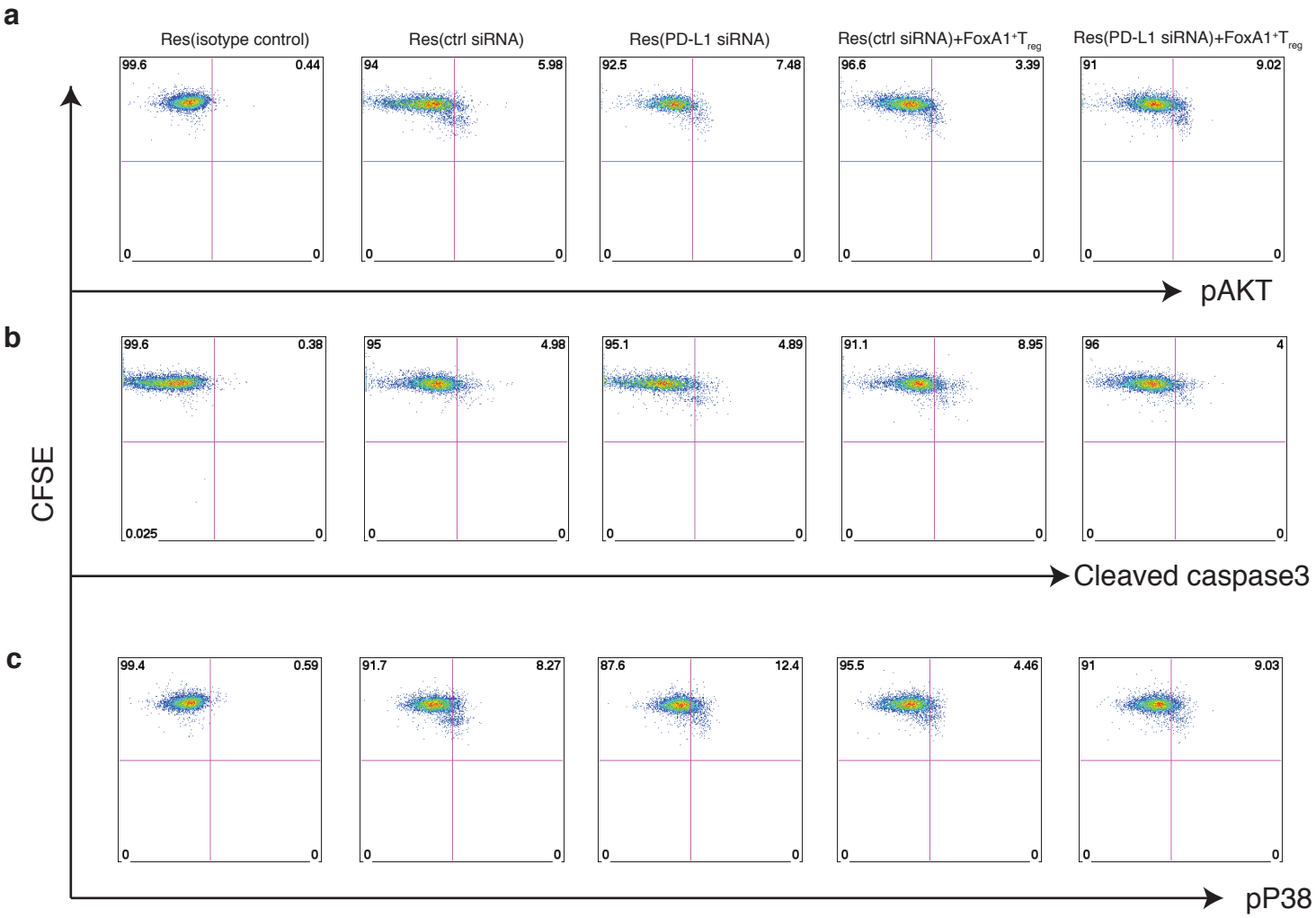


Supplementary Figure 8. Human iFoxA1^{+}T$_{reg}$ cells suppress effector function of APCs by inhibiting their pro-inflammatory cytokines production.

MACS purified APCs from healthy blood donors were treated with LPS for 2 days prior to co-culture with iFoxA1^{+}T$_{reg}$ cells. After 24 hours of co-culture of APCs with FoxA1^{+}T$_{reg}$ cells, intracellularly stained cytokines were gated on APCs (HLA-DR^{+}TCR^{-}), supernatant were analyzed for different cytokines using ELISA. **(a)** One representative of FACS dot plots show IL-10, **(b)** IL-12, and **(c)** IL-17 productions by ELISA. Bars represent mean ± s.d. *$P < 0.05$. Student's unpaired *t* test were used for analysis, n=3.

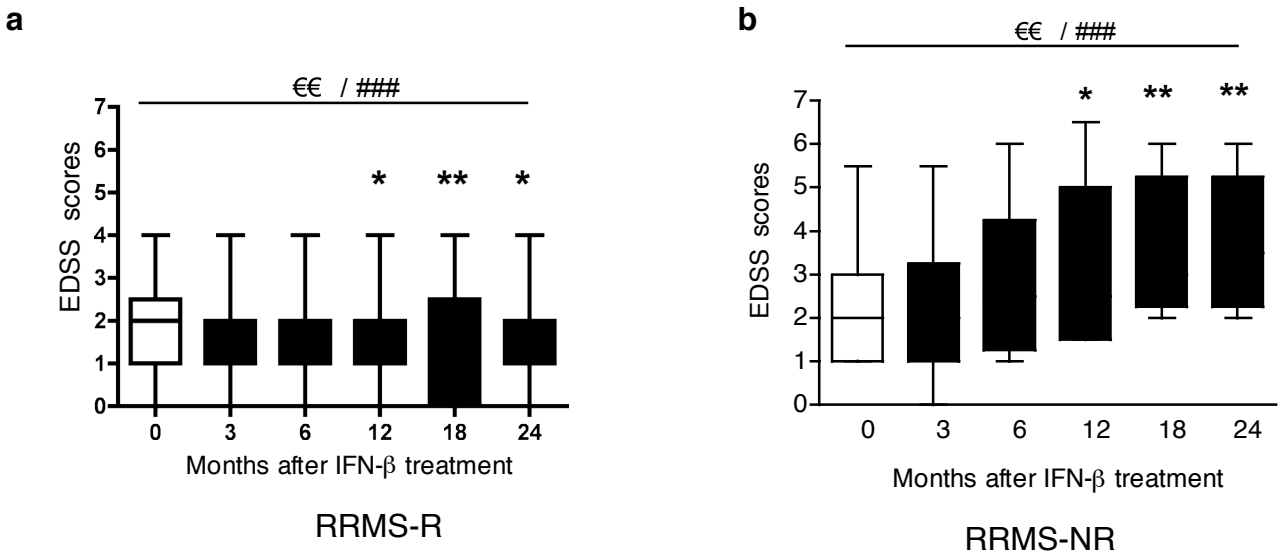


Supplementary Figure 9. Rescue of FoxA1^{+}T$_{reg}$ phenotype by ectopic expression of FoxA1 confirms specificity of siRNAs targeting. (a) Scheme for gene targeting and rescue strategy depicts pcDNA3.1 FoxA1, FoxA1siRNAs and amplified regions by indicated PCR primers. (b) Murine purified CD4^{+} T cells were transfected with siRNAs. Efficiency of FoxA1 KD was confirmed by qPCR after 72 h of transfection, using 4 different siRNAs and a smartpool FoxA1 siRNA (4 siRNA pooled). (c) Purified CD4^{+} T cells were transfected by pcDNA3.1FoxA1. pcDNA3.1 vector was used as a control. qPCR was performed for the indicated regions. (d-e) Ectopic FoxA1 is not affected by siRNAs targeting 3'UTRs. Purified CD4^{+} T cells were transfected with a smartpool FoxA1 siRNAs for 24 h, followed by transfection with pcDNA3.1FoxA1 for additional 48h. (d) Expression levels of FoxA1 gene were measured by qPCR which indicates that FoxA1 siRNAs are specifically targeting and deleting endogenous FoxA1, (e) while pcDNA3.1FoxA1 is rescuing FoxA1 determined by positive ORF primer readout but not 3'UTR primers. Data are means±s.d. from duplicates.



Supplementary Figure 10. Human iFoxA1^{+}T$_{reg}$ cells suppress responder T cells via PD-L1-PD-1 mediated inhibition of pAKT, pP38 and upregulation of cleaved caspase3.

Purified CD4^{+} T cells from healthy blood donors were treated *in vitro* with hIFN-β1000U/ml for 2 days and FoxA1^{+}T$_{reg}$ cells were purified. CFSE-labeled purified CD4^{+} T cells from the same healthy blood donors were transfected either with control siRNA (UNC) or PD-1 siRNA (PD-1KD) for 3 days, then activated with plate-coated anti-CD3 antibody for 24 h (responder T cells). Control siRNA and PD-1 siRNA silenced responder T cells were co-cultured with purified FoxA1^{+}T$_{reg}$ for additional 24 h. A representative CSFE FACS histogram shows that FoxA1^{+}T$_{reg}$ inhibit proliferation of **(a)** phosphorylated AKT and **(b)** cleaved caspase 3. **(c)** Phosphorylated p38 in responder T cells in a PD-1 dependent manner.



Supplementary Figure 11. Changes on EDSS scores over time in RRMS patients treated with IFN-β.

(**a)** EDSS scores in RRMS-R patients before (baseline) and after 24 months of treatment with IFN-β show no progression on neurological disability during the follow-up period. **(b)** EDSS scores in RRMS-NR patients indicate progression on neurological disability over time. n=9-15 per group. One-way ANOVA with repeated measures (€€$P < 0.01$, €€€$P < 0.001$), post-test Dunnett's Multiple Comparison (*$P < 0.05$, **$P < 0.01$) and linear trend tests (###$P < 0.001$) were used. The linear trend test revealed a $P < 0.001$ with positive slope (increasing EDSS) in the RRMS-NR and $P < 0.001$ with a negative slope (decreasing EDSS) in the RRMS-R group.

Supplementary Tables

Supplementary Table I: *Ifnb*$^{-/-}$ mice develop more severe relapsing EAE than WT mice.

Group	Relapsing frequency	Recovered frequency	Mean max CS±SD	Max CS	Mean cumulative CS	Incidence
WT	10/41 = 24%	30/41 = 74%	1.9±1.3	4	17	71% (15/21)
Ifnb$^{-/-}$	10/20 = 50%*	9/20 = 45%*	3.6±0.9***	5	38	95%* (19/20)

Relapsing frequency= Number of relapsing mice are calculated from total number of mice. Recovery frequency = Number of recovered mice per total number of mice.

*p<0.05, *Chi*-square test indicating *Ifnb*$^{-/-}$ mice had higher relapsing frequency, higher recovered frequency and higher incidence in comparison with WT mice.

Mean cumulative CS (clinical score): was calculated by summing up each individual score registered during the follow-up period till day 51 divided by the number of mice per group.

Mean max CS, ***p<0.001, Mann-Whitney test.

Supplementary Table II. Differential expression of an array of selected genes in $CD4^{hi}Pd\text{-}l1^{hi}$ T cells versus encephalitogenic (EncT cells/$MBP_{89\text{-}101}$-specific) progenitor $CD4^{+}$T cells.

Gene Name	Gene Symbol	Probe Set ID	*P* Value	Fold Change $FoxA1^{+}T_{regs}$ vs EncT
Pro-inflammatory cytokines/receptors				
Tumor necrosis factor	Tnf	1419607_at	0.012	0.45
Interleukin 1 alpha	Il1a	1421473_at	0.0192	0.25
Interleukin 1 beta	Il1b	1449399_a_at	0.000128	0.36
Interleukin 5	Il5	1450550_at	0.0158	0.47
Interleukin 7 receptor Interleukin 7 receptor	Il7r Il7r	1448576_at 1448575_at	0.00693 0.0017	0.56 0.59
Interleukin 13	Il13	1420802_at	0.0211	0.42
Interleukin 16	Il16	1448686_at	0.0059	0.67
Interleukin 17 receptor D	Il17rd	1429893_at	0.0208	0.64
Interleukin 18 receptor 1	Il18r1	1421628_at	0.0415	0.42
CD & adhesion molecules				
CD47 antigen (Rh related, integrin-associated signal transducer)	cd47	1458634_at	0.00524	2.30
CD69 antigen	Cd69	1428735_at	0.0321	1.82
Cerebral endothelial cell adhesion molecule 1	Cercam1	1435345_at	0.016	0.71
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	Plod3	1415901_at	0.0234	0.69
Procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha 1 polypeptide	P4ha1	1426519_at	0.0457	0.65
Transcription factors				
Forkhead box A1	FoxA1	1418496_at	0.00105	846.0

Supplementary Table III: Top Canonical Pathways overrepresented in nFoxA1^{+}T$_{reg}$ cells versus EncT cells.

	GENE SET	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	PID_HNF3APATHWAY	38	0.66	1.85	0.002	0.509	0.382	1664
2	PID_HNF3BPATHWAY	38	0.63	1.75	0.009	0.897	0.794	1871
3	REACTOME_M_G1_TRANSITION	67	0.56	1.71	0	0.914	0.919	8324
4	REACTOME_DNA_STRAND_ELONGATION	25	0.67	1.7	0.013	0.77	0.936	6280
5	REACTOME_TRNA_AMINOACYLATION	30	0.65	1.69	0.004	0.657	0.95	6124
6	REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	101	0.52	1.69	0	0.557	0.953	8988
7	REACTOME_P130CAS_LINKAGE_TO_MAPK_SIGNALING_FOR_INTEGRINS	15	0.72	1.68	0.013	0.528	0.967	2431
8	REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	19	0.7	1.68	0.011	0.472	0.973	4606
9	KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	29	0.65	1.68	0.009	0.429	0.975	6124
10	KEGG_DNA_REPLICATION	32	0.61	1.67	0.017	0.408	0.982	6280
11	REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	27	0.63	1.66	0.013	0.397	0.986	6415
12	REACTOME_MITOTIC_M_M_G1_PHASES	137	0.48	1.66	0.002	0.393	0.99	8324
13	REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM	43	0.58	1.65	0.013	0.365	0.991	8719
14	REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	21	0.66	1.65	0.017	0.359	0.993	7160
15	PID_CD8TCRDOWNSTREAMPATHWAY	54	0.55	1.65	0.004	0.346	0.994	535

Top 15 canonical pathways (gene sets) overrepresented in neuron-induced (n)FoxA1^{+}T$_{reg}$ cells compared with their progenitor EncT cells using GSEA analysis. GSEA analysis was quantile normalized and summarized for each comparison using justPlier implementation of Plier algorithm in R. ES=enrichment score, NES= normalized enrichment score, NOM p-val= nominal p value, FDR q-val = False Discovery Rate, FWER p-val = family-wise error rate, RANK AT MAX= position in the ranked list at which the maximum enrichment score occurred.

Supplementary Table IV: Top Canonical pathways overrepresented in T_{reg} versus T_n cells.

	GENE SET	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	PID_PLK1_PATHWAY	40	0.65	1.92	0	0.127	0.1	3480
2	BIOCARTA_AMI_PATHWAY	19	0.72	1.84	0.004	0.24	0.317	3391
3	REACTOME_IL_RECEPTOR_SHC_SIGNALING	25	0.69	1.83	0.002	0.174	0.339	1334
4	REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	211	0.48	1.83	0	0.143	0.359	2371
5	REACTOME_INTERFERON_GAMMA_SIGNALING	40	0.61	1.79	0	0.192	0.532	5195
6	BIOCARTA_IL2_PATHWAY	21	0.69	1.79	0.002	0.173	0.564	220
7	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	202	0.47	1.77	0	0.176	0.618	2281
8	PID_IL2_1PATHWAY	52	0.58	1.76	0	0.171	0.651	4621
9	REACTOME_MITOTIC_PROMETAPHASE	66	0.54	1.75	0	0.174	0.701	2104
10	REACTOME_IL_2_SIGNALING	38	0.6	1.75	0.004	0.163	0.717	1334
11	PID_CD8TCRDOWNSTREAMPATHWAY	54	0.55	1.73	0.002	0.2	0.827	2234
12	BIOCARTA_MYOSIN_PATHWAY	30	0.63	1.72	0.004	0.187	0.831	1595
13	REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	44	0.57	1.72	0.004	0.178	0.837	2281
14	REACTOME_DNA_REPLICATION	154	0.47	1.71	0	0.199	0.89	3557
15	REACTOME_GROWTH_HORMONE_RECEPTOR_SIGNALING	20	0.67	1.7	0.006	0.21	0.907	3368

Top 15 canonical pathways (gene sets) overrepresented in T_{regs} compared with their control T_n cells (analysis based on Samstein et al. 2012 Cell). GSEA analysis, ES=enrichment score, NES= normalized enrichment score, NOM p-val= nominal p value, FDR q-val = False Discovery Rate, FWER p-val = family-wise error rate, RANK AT MAX= position in the ranked list at which the maximum enrichment score occurred.

Supplementary Table V. FACS characterization of murine FoxA1^{+}T$_{reg}$ cells.

Negative expression	Positive expression
TGF-β	CD4^{hi}
Ctla-4	Pd-l1^{hi}
FoxP3	CD25
Fas	Pd-1
Fasl	CD45Rb
IL-17	CD69
IL-4	CD47
IL-10	FoxA1

Supplementary Table VII. nFoxA1^{+}T$_{reg}$ cells suppress EAE in *Ifnb*$^{-/-}$ mice.

Groups	Treatments	Mean cumulative CS	Max CS	Mean Max CS±SD[1]	Incidence	Day of onset
WT	+ 2×10^{6} control T cells	5.7	2	0.5±0	25%	9
Ifnb$^{-/-}$	+ 2×10^{6} control T cells	12.2	5	1±0.5	60%	9
Ifnb$^{-/-}$	+ 2×10^{6} nFoxA1^{+}T$_{regs}$	6	2	0.5±0	25%	11

Adoptive EAE was established using 2 × 10^{6} encephalitogenic MBP$_{89\text{-}101}$-reactive T cells (EncT-cells). *n* =4-5/group. Control (CD4^{+} T-cells) or nFoxA1^{+}T$_{reg}$ (CD4^{hi}Pd-l1^{hi}) T cells were co-transferred with EncT cells. [1] among sick mice.

Mean cumulative CS (clinical score): was calculated by summing up each individual score registered during the follow-up period until day 24 divided by the number of mice per group.

Supplementary Table VIII. Top Transcription Factors overrepresented in iFoxA1^{+}T$_{reg}$ among commonly regulated genes by different FoxA1^{+}T$_{reg}$ cells.

ID	SYMBOLL	logFC	AveExpr	t	P.Value	adj.P.Val	B
1429427_s_at	Tcf7l2	14,58	-2,286	7,19	0,0001	0,0001	0,58
1449134_s_at	Spic	13,31	-1,215	4,42	0,003	0,003	-2,55
1460038_at	Pou3f1	13,23	-2,951	6,88	0,0002	0,0002	0,28
1434102_at	Nfib	12,04	-4,194	5,49	0,0009	0,0009	-1,22
1451716_at	Mafb	11,58	0,353	3,52	0,009	0,0097	-3,80
1451506_at	Mef2c	10,04	1,341	3,39	0,011	0,0115	-3,98
1451507_at	Mef2c	9,34	2,033	2,87	0,023	0,0237	-4,75
1419874_x_at	Zbtb16	9,02	-2,311	3,32	0,012	0,0127	-4,09
1429428_at	Tcf7l2	8,25	1,179	4,26	0,003	0,003	-2,76
1436515_at	Bach2	7,50	4,749	7,31	0,0001	0,0001	0,69
1460591_at	Esr1	7,23	0,942	2,58	0,036	0,036	-5,20
1421027_a_at	Mef2c	7,10	3,983	2,79	0,026	0,026	-4,87
1448890_at	Klf2	6,98	5,979	22,80	8.281e-08	8.281e-08	8,96
1418496_at	Foxa1	6,87	-0,652	3,05	0,018	0,018	-4,49
1433939_at	Aff3	6,50	2,262	3,89	0,005	0,005	-3,27
1460407_at	Spib	6,49	8,848	39,25	1.924e-09	1.924e-09	12,57
1433471_at	Tcf7	6,44	5,5346	10,70	1.396e-05	1.396e-05	3,43
1425793_a_at	Rorc	-6,59	2,979	-2,84	0,024	0,0249	-4,80
1418811_at	Barhl1	-6,63	0,009	-3,52	0,009	0,009	-3,80
1419576_at	Hoxb13	-7,00	-0,930	-3,39	0,011	0,011	-3,99
1438737_at	Zic3	-7,07	-1,030	-3,54	0,009	0,009	-3,76
1426637_a_at	Six3	-7,31	-3,480	-2,65	0,032	0,032	-5,09
1427697_a_at	Trp73	-7,54	3,543	-3,06	0,018	0,018	-4,48
1422336_at	Hoxa13	-7,54	0,815	-2,88	0,023	0,023	-4,74
1424035_at	Rora	-7,54	3,021	-3,17	0,015	0,015	-4,30
1422267_at	Foxb2	-7,85	0,428	-3,35	0,012	0,012	-4,04
1439193_at	Prrxl1	-8,53	2,042	-2,38	0,048	0,048	-5,49
1422312_a_at	Neurog3	-8,86	0,989	-3,54	0,009	0,009	-3,77
1427538_at	Zfp369	-10,03	-2,116	-12,52	4.906e-06	4.906e-06	4,59

Supplementary Table IX: Top Canonical Pathways overrepresented in nFoxA1^{+}T$_{reg}$ among commonly regulated genes by different FoxA1^{+}T$_{reg}$ cells.

	GENE_SET	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	**PID_AP1_PATHWAY**	6	0.81	1.81	0.007	0.285	0.247	86
2	**PID_HNF3APATHWAY**	4	0.83	1.64	0.021	1	0.914	71
3	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR _CARDIOMYOPATHY_ARVC	6	0.74	1.6	0.037	1	0.969	136
4	**REACTOME_ION_CHANNEL_TRANSPORT**	4	0.82	1.6	0.029	0.934	0.975	140
5	**KEGG_MELANOGENESIS**	3	0.88	1.6	0.01	0.751	0.975	92
6	PID_P53DOWNSTREAMPATHWAY	5	0.76	1.58	0.043	0.76	0.985	3
7	PID_SHP2_PATHWAY	5	0.73	1.55	0.056	0.876	0.994	114
8	**KEGG_MAPK_SIGNALING_PATHWAY**	13	0.58	1.53	0.061	0.903	0.998	18
9	REACTOME_ION_TRANSPORT_BY_P_TYPE_ATPASES	3	0.82	1.49	0.066	1	1	140
10	**KEGG_ARACHIDONIC_ACID_METABOLISM**	4	0.74	1.45	0.096	1	1	202
11	KEGG_CARDIAC_MUSCLE_CONTRACTION	4	0.74	1.44	0.109	1	1	140
12	**KEGG_GNRH_SIGNALING_PATHWAY**	4	0.72	1.39	0.137	1	1	92
13	KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	5	0.68	1.38	0.124	1	1	154
14	**REACTOME_GLYCEROPHOSPHOLIPID_BIOSYNTHESIS**	3	0.75	1.37	0.142	1	1	18
15	PID_CD8TCRDOWNSTREAMPATHWAY	7	0.59	1.36	0.157	1	1	81
16	PID_NFAT_TFPATHWAY	7	0.58	1.35	0.183	1	1	41
17	REACTOME_CELL_SURFACE_INTERACTIONS _AT_THE_VASCULAR_WALL	9	0.54	1.33	0.205	1	1	218
18	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	5	0.61	1.31	0.212	1	1	136
19	KEGG_ADHERENS_JUNCTION	3	0.71	1.31	0.177	1	1	114
20	KEGG_LEISHMANIA_INFECTION	3	0.72	1.3	0.166	1	1	102

Top 20 canonical pathways overrepresented in nFoxA1^{+}T$_{reg}$ compared with their progenitor EncT cells, among genes commonly regulated by iFoxA1^{+}T$_{reg}$ and nFoxA1^{+}T$_{reg}$ vs. EncT cells were analyzed using GSEA analysis. In bold: common pathways found among the top 20 canonical pathways in nFoxA1^{+}T$_{reg}$ and IFNb-induced/iFoxA1^{+}T$_{reg}$. GSEA analysis was quantile normalized and summarized for each comparison using justPlier implementation of Plier algorithm in R. ES=enrichment score, NES= normalized enrichment score, NOM p-val= nominal p value, FDR q-val = False Discovery Rate, FWER p-val = family-wise error rate, RANK AT MAX= position in the ranked list at which the maximum enrichment score occurred.

Supplementary Table X: Top Canonical Pathways overrepresented in IFNβ-induced/iFoxA1^{+}T$_{reg}$ among commonly regulated genes by different FoxA1^{+}T$_{reg}$ cells.

	GENE_SET	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	**PID_AP1_PATHWAY**	6	0.78	1.74	0.013	0.428	0.348	48
2	KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	7	0.73	1.7	0.017	0.379	0.534	30
3	**KEGG_MELANOGENESIS**	3	0.93	1.67	0	0.356	0.673	57
4	KEGG_GAP_JUNCTION	3	0.92	1.63	0.004	0.396	0.818	48
5	**KEGG_GNRH_SIGNALING_PATHWAY**	4	0.83	1.62	0.019	0.385	0.881	31
6	REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	5	0.72	1.54	0.05	0.688	0.987	80
7	PID_CXCR3PATHWAY	3	0.87	1.53	0.031	0.618	0.989	24
8	REACTOME_INNATE_IMMUNE_SYSTEM	9	0.6	1.48	0.07	0.845	1	37
9	KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	3	0.81	1.47	0.045	0.813	1	35
10	**KEGG_ARACHIDONIC_ACID_METABOLISM**	4	0.76	1.47	0.05	0.735	1	29
11	**REACTOME_ION_CHANNEL_TRANSPORT**	4	0.73	1.46	0.077	0.73	1	132
12	REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS	13	0.52	1.45	0.077	0.699	1	27
13	**PID_HNF3APATHWAY**	4	0.73	1.45	0.087	0.663	1	209
14	KEGG_LONG_TERM_DEPRESSION	4	0.74	1.45	0.064	0.628	1	10
15	KEGG_GLYCEROPHOSPHOLIPID_METABOLISM	3	0.81	1.44	0.067	0.613	1	10
16	KEGG_LONG_TERM_POTENTIATION	3	0.8	1.42	0.083	0.67	1	31
17	REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	7	0.61	1.39	0.101	0.765	1	80
18	**REACTOME_GLYCEROPHOSPHOLIPID_BIOSYNTHESIS**	3	0.77	1.38	0.1	0.778	1	10
19	**KEGG_MAPK_SIGNALING_PATHWAY**	13	0.5	1.38	0.124	0.752	1	65
20	REACTOME_TOLL_RECEPTOR_CASCADES	4	0.69	1.37	0.127	0.746	1	37

Top 20 canonical pathways overrepresented in iFoxA1^{+}T$_{reg}$ compared with EncT cells, among genes commonly regulated by i FoxA1^{+}T$_{reg}$ and nFoxA1^{+}T$_{reg}$ vs. EncT cells were analyzed using GSEA analysis. In bold: common pathways found among the top 20 canonical pathways in nFoxA1^{+}T$_{reg}$ and IFNb-induced/iFoxA1^{+}T$_{reg}$. GSEA analysis was quantile normalized and summarized for each comparison using justPlier implementation of Plier algorithm in R. ES=enrichment score, NES= normalized enrichment score, NOM p-val= nominal p value, FDR q-val = False Discovery Rate, FWER p-val = family-wise error rate, RANK AT MAX= position in the ranked list at which the maximum enrichment score occurred.

Supplementary Table XI. FoxA1^{+}T$_{reg}$ cells suppress adoptive EAE in *Ifnb*$^{-/-}$ mice mediated by FoxA1 and Pd-l1 signaling.

Groups	Treatments	Mean cumulative CS	Max CS	Mean Max CS±SD[1]	Incidence	Day of onset
ctrl-T (ctrl siRNA +pcDNA3.1)	+ 5×10^6 activated T cells	25	4	2.25±1.1	54.5% (*n*=12/22)	9
iFoxA1^{+}T$_{reg}$ (ctrl siRNA)	+ 5×10^6 iFoxA1^{+}T$_{reg}$	1.7	3	NA	11%* (*n*=1/9)	34
iFoxA1^{+}T$_{reg}$ (FoxA1 siRNA)	+ 5×10^6 iFoxA1^{+}T$_{reg}$ (FoxA1 siRNA)	16	4	2.16±1.2	46% (*n*=6/13)	13
iFoxA1^{+}T$_{reg}$ (Pd-l1 siRNA+anti-Pd-l1)	+ 5×10^6 iFoxA1^{+}T$_{reg}$ (Pd-l1 siRNA+anti-Pd-l1)	36	4	3.75±0.4	50% (*n*=4/8)	14
FoxA1^{+}T$_{reg}$ (FoxA siRNA+pcDNA3.1FoxA1)	+ 5×10^6 FoxA1^{+}T$_{reg}$ (FoxA siRNA+pcDNA3.1FoxA1)	3	1	NA	10%** (*n*=1/10)	12

Adoptive EAE was established by twice transferring 5-10 × 10^6 MOG$_{35-55}$-reactive EncT cells (day 0 and d 14). *n*= 8-22. Control (CD3 activated T cells, with ctrl siRNA+pcDNA3.1), iFoxA1^{+}T$_{reg}$ (IFN-β treated 48 hours, with ctrl siRNA), iFoxA1^{+}T$_{reg}$ (FoxA1 siRNA KD for 24 hour + IFN-β treated 48 hours), iFoxA1^{+}T$_{reg}$ (Pd-l1 siRNA KD for 24 hour + IFN-β treated 48 hours) and FoxA1^{+}T$_{reg}$ (FoxA1 siRNA+pcDNA3.1 FoxA1) cells were co-transferred each time with EncT cells. The results are sum of 3 independent experiments.

[1]among sick mice, NA = not applicable since only one mice developed disease in these groups which preclude statistic analysis.

**p*<0.05, ** *p*<0.01, *Chi*-square test indicating significant low incidence in these groups.

Mean cumulative CS (clinical score): was calculated by summing up each individual score registered during the follow-up period until day 40 divided by the number of mice per group.

Statistics for mean clinical score comparison by one-way Anova and multiple comparisons (*** *p*<0.001).

Tac vs. iFoxA1^{+}T$_{reg}$	***
Tac vs. iFoxA1^{+}T$_{reg}$ (FoxA1siRNA)	ns
Tac vs. iFoxA1^{+}T$_{reg}$ (Pd-l1 siRNA+anti-Pd-l1)	ns
Tac vs. FoxA1^{+}T$_{reg}$ (FoxA1siRNA+pcDNA3.1 FoxA1)	***
iFoxA1^{+}T$_{reg}$ vs. iFoxA1^{+}T$_{reg}$ (FoxA1siRNA)	***
iFoxA1^{+}T$_{reg}$ vs. iFoxA1^{+}T$_{reg}$ (Pd-l1 siRNA+anti-Pd-l1)	***
iFoxA1^{+}T$_{reg}$ vs. FoxA1^{+}T$_{reg}$ (FoxA1siRNA+pcDNA3.1 FoxA1)	ns

Supplementary Methods

Mice

Ifnb$^{-/-}$ mice and *Ifnb*$^{+/-}$, WT littermates in C57BL/10.RIII or C57BL/6 were bred and kept at conventional animal facilities at the University of Copenhagen. *Ifnar*$^{-/-}$ mice were from B & K Universal, UK. NOG (NOD.Cg*Prkdc*^{scid}*il2rg*^{tm1sug}/jicTac) and OT-II (B6.129S6-*Rag2*^{tm1Fwa}Tg(TcraTcrb)425 Cbn) mice were purchased from Taconic, Denmark and The Jackson Laboratory, USA, respectively.

Experiments were approved and performed in accordance with the national ethical committee (Animal Experiments Inspectorate under Danish Ministry of Food, Agriculture and Fisheries, The Danish Veterinary and Food Administration), approval number 2007/561-1364.

EAE induction and clinical evaluation

1) Active EAE

A) C57BL/10.RIII EAE: *Ifnb*$^{-/-}$ C57BL/10.RIII, *Ifnb*$^{+/-}$ C57BL/10.RIII heterozygote (HT) or WT littermates were used for EAE induction. Gender– and age–matched mice were used in all experiments. As no statistical differences were found between HT and WT littermates, unless specified, we have referred to them as WT. Each animal was subcutaneously immunized in the base of the tail with 100 µl of a 1:1 emulsion of 250 µg of MPB_{89-101} (Schafer-Copenhagen) in PBS and CFA containing 500 µg of *Mycobacterium tuberculosis* H37Ra (Difco). An i.p. injection of 500 ng of pertussis toxin/PT (*Bordetella pertussis*; Sigma-Aldrich) dissolved in 100 µl of PBS was given on the day of immunization (day 0) and at day 2. Mice were blindly scored for clinical signs of EAE every day.

B) C57BL/6 EAE: *Ifnb*$^{-/-}$ C57BL/6, WT littermates were used for EAE induction. Gender– and age–matched mice were used in all experiments. Each animal was subcutaneously immunized in the base of the tail with 100 µl of a 1:1 emulsion of 150 µg of MOG_{35-55} (Schafer-Copenhagen) in PBS and CFA containing 500 µg of *Mycobacterium tuberculosis* H37Ra (Difco). An i.p. injection

of 500 ng of pertussis toxin/PT (*Bordetella pertussis*; Sigma-Aldrich) dissolved in 100 µl of PBS was given on the day of immunization (day 0). Mice were blindly scored for clinical signs of EAE every day. For some EAE experiments, mice were received i.p. injection of mouse recombinatant IFN-β (5000U) at day 0, day 7 and day 14 post immunization.

2) Adoptive Transfer EAE

A) C57BL/6 background: For adoptive transfer EAE, C57BL/6 and *Ifnb*$^{-/-}$ C57BL/6 mice were used. Gender– and age–matched mice were used in all experiments. Each mouse was irradiated (500 rad) and i.v. injected in the tail vein with a spleen cell suspension of 5-10 x 10^6 from MOG_{35-55}-immunized WT mice twice (day 0 and 14). At day 0 and day 2, each animal was given an i.p. injection of 500 ng of pertussis toxin. At day 0 and 14, each mouse received co-transfer (i.v. injection) of either 5 x 10^6 in 100 µl of PBS of control purified $CD4^+$ T cells, or iFoxA1$^+$$T_{reg}$ cells (after 48 h of IFN-β treatment), or iFoxA1$^+$$T_{reg}$ (FoxA1 siRNA) cells (KD with FoxA1 siRNA for 24 h, then 48 h of IFN-β treatment), iFoxA1$^+$$T_{reg}$ (Pd-l1 siRNA+anti-Pd-l1) cells (KD with Pd-l1 siRNA for 24 h, then 48 h of IFN-β treatment + anti-Pd-l1) or FoxA1$^+$$T_{reg}$ ($CD4^+$ T cells treated with FoxA1 siRNA for 24 h and then transfected with pcDNA3.1foxa1 for additional 48h). In some experiments mice were treated with purified equal number of natural T_{reg} cells (using mouse $CD4^+CD25^+$ Regulatory T cell isolation Kit, Miltenyi biotech) or TGF-β-induced T_{reg} cells upon adoptive transfer of encephalitogenic cells. Mice were blindly scored for clinical signs of EAE every day.

B) C57BL10/RIII: C57BL/10.RIII WT and *Ifnb*$^{-/-}$ C57BL/10.RIII mice were used. Male age matched mice were used in all experiments. Each mouse was irradiated (500 rad) and injected in the tail vein with a cell suspension of 2 x 10^6 MBP_{89-101}-specific T cells derived from immunized WT-mice and activated with the antigen for several passage (MBP_{89-101}-EncT cell line). At day 0 and day 2, each animal was given an i.p. injection of 500 ng of pertussis toxin. At day 0, each mouse

received co-transfer of either 2 x 10^6 in 300 µl of PBS of $MBP_{89–101}$–specific T cells, or $nFoxA1^+T_{reg}$ cells purified from co-culture of neurons and MBP_{89-101}-EncT-cell lines. Mice were blindly scored for clinical signs of EAE every day.

Accoring to lab experience and pilot study, minimum 5 mice per group is a criteria to choose for each EAE, Two or three independent EAE experiments were repeated. Regarding their age and gender, Mice were equally allocated to experimental groups.

Clinical scoring for EAE was as follows: 0, no disease; 1, limp tail; 2, limp tail and ataxia as well as hind limb weakness/unsteady walk; 3, both hind limbs affected; 4, complete paralysis of hind limbs until hips; 5, moribund or dead.

Relapses were defined as improvement in clinical score after the acute phase of disease by at least one point on the scoring scale (remission phase), followed by a worsening of disease of a minimum of one point on the scoring scale. The total recovery frequency was defined as total recovery/remission after acute phase of disease or relapses to the clinical score of zero, with or without subsequent relapses. The accumulative score was calculated as the sum of all clinical scores of each individual mouse during the entire observation period and is thus equivalent to the area under the curve. The maximum clinical score was defined as the highest score that each individual reached during the whole experiment. Incidence was defined as the percentage of animals that developed EAE during the experimental period. Mean score over experimental period was calculated for each animal as the sum of all individual scores divided by the number of measurements. EAE scoring was conducted blindly. All animals, including severely EAE-affected animals, had access to food and water throughout the experiments.

DTH response

Mice aged 9–15 weeks were immunized with 250 µg of MBP_{89-101} emulsified in 50 µl of PBS and 50 µl of CFA. At day 13 after immunization, Male mice were injected with 100 µg of MBP_{89-101} in PBS and $FoxA1^+T_{reg}$ cells ($3x10^4$ cells/ear) in the right ear or 100 µg of MBP_{89-101} in

PBS and control T cells ($MBP_{89–101}$–specific T cells, $3x10^4$ cells/ear) in the left ear. Control male mice received an injection of 100 μg of MBP_{89-101} in PBS in the left ear and PBS with control T cells ($3x10^4$ cells/ear) in the right ear. After 24 h and 48 h, the DTH response was measured as the difference in swollenness / thickness (mm) of the right and left ears. Data for the control T-cell group are presented as: (ear thickness after injection with MBP_{89-101} and control T cells) - (ear thickness after injection with MBP_{89-101}). Data for the $FoxA1^+T_{reg}$ cells group are presented as: (ear thickness injected with MBP_{89-101} and $FoxA1^+T_{reg}$ cells) - (ear thickness after injection with MBP_{89-101}).

Preparation of CNS infiltrating cells

At the indicated times after active EAE induction, the brain was rapidly removed, and hemispheres were separated, freed from meninges, and placed into 15 ml of ice-cold PBS containing 0.2% BSA (Sigma), 0.01 mol/L EDTA (BTH), and 10 mg/ml deoxyribonuclease I (Sigma). Hemispheres were disrupted in a glass homogenizer and passed through a 40-μm nylon cell strainer (Becton Dickinson). The suspension was centrifuged at 400 x g for 10 min at room temperature, and the pellet suspended in 4 ml of 30% Percoll (Amersham Pharmacia Biotech) and overlaid on a gradient of 3.5 ml of 37% and 3.5 ml of 70% Percoll solution, prepared by dilution in HBSS. The gradient was centrifuged at 500 x g for 20 min at room temperature and 5 ml cells were collected from the 37% to 70% interface and washed once with PBS containing 10% FCS.

Neuronal cultures

Complete neuron basal medium (21103-049, Invitrogen) was prepared by adding 2% B27 (17504-044, Gibco), 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.5 mM L-glutamine (G2150, Sigma-Aldrich). 96-well culture plates were coated with a poly-D-lysine solution (70 μg/ml, P7280, Sigma-Aldrich) in sterile distilled water and incubated for at least 30 min and then washed with sterile distilled water.

A 7-day-old mouse pup was decapitated, the head immediately put in a 50 ml tube with Hank's balanced salt solution (HBSS, 14170, Gibco) on ice. It was then transferred to a Petri-dish (containing 1 ml HBSS) in the sterile hood and dissected under a microscope. The cerebellum was carefully dissected out and meninges were taken away while cerebellum kept intact. A single cell suspension was prepared by adding a trypsin (T4174-100ml, Sigma-Aldrich) solution to a final concentration of 200 μg/ml. Thereafter DNAse (D5024-150KU, Sigma-Aldrich) and FCS were added to final concentrations of 0.12% and 0.5% respectively and incubated for 8 min. Finally the solution was mechanically dissociated to single cell levels using a fire constricted Pasteur pipette. The cells were seeded at a concentration of $4x10^5$ cells/ml with complete neuron basal medium in 96-well culture plate. The purity of culture was measured using FACS staining. More than 95% purity was achieved determined by Neurofilament 200 staining (N4142, Sigma-Aldrich 1:150).

Establishment of myelin-specific T cell lines

To generate T cell lines, 8-12 weeks old male B10RIII were immunized in the flank and tail base with 200 μl of a 1:1 emulsion of 250 μg of MBP_{89-101} in PBS and CFA containing *Mycobacterium tuberculosis* H37Ra (Difco). Draining lymph nodes were collected 10 days after immunization, and a single cell suspension prepared in PBS by passing through a sieve. Cells were washed and suspended in DMEM with Glutamax-1 (Gibco) supplemented with 10 mM HEPES buffer, 10% heat-inactivated FCS (Sigma), 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 50 μM 2-mercaptoethanol, to make complete DMEM (cDMEM). Cells were cultured as $5x10^5$ cells in round-bottomed 96-well culture plates (Nunc) in 200 μl of cDMEM, in a humidified 37°C atmosphere containing 5% CO_2. T-cells were stimulated for 4 days with 50 μg of MBP_{89-101}. After a resting phase of 8 days in media supplemented with 800 pg/ml of IL-2 (obtained from the supernatant of an IL-2 transfected X63 cell line), T cells were re-stimulated with 20 μg of peptide and APCs. APC were generated from spleen cells of syngeneic mice, prepared as described above for lymph nodes with an additional 0.84% NH_4Cl treatment to lyse red blood cells, and irradiated

with 3,000 rad before being used at a concentration 10 times higher than the T cells. To expand highly specific T-cell lines, stimulation was repeated at intervals of 10-30 days. Between stimulations, the antigen-containing media was removed and the T cells kept in a resting state in cDMEM supplied with IL-2. The media was changed every fourth day. In all experiments, T-cell lines had gone through a total of 4–10 stimulation rounds.

Co-culture of neuronal cells with T cells

Cerebellar granular neurons (CGNs) were seeded at $4x10^5$ cells/ml in 96- or 24-well plates with complete neuron basal medium for at least 3 days. T-cell lines were re-stimulated with antigen and APCs for 48 h and are referred to as activated T cells. Neurons and activated T cells were washed twice and co-cultured at a 1:1 ratio for 24 h as previously described[1].

Patients

Totally 50 individuals were included, 28 patients with RRMS treated with IFN-β and 22 healthy controls were included in the current study. Informed patients consent is received.

24 RRMS patients treated with IFN-β were included in the first study. Of these, 15 were good IFN-β responder RRMS (7 females/8 males; mean age [standard deviation]: 34.3 years [7.8]) and 9 RRMS-NR (7 females/1 males; mean age: 37.1 years [8.6]). Patients with RRMS were classified as good responders to IFN-β based on the absence of relapses and no progression on the EDSS score during the first two years of treatment [2]. Patients were labeled as RRMS-NR when there was presence of relapses during the follow-up period; one or more relapses and an increase of at least 1 point in the EDSS score that persisted for at least two consecutive scheduled visits separated by a 6-month interval[2]. Classification of MS patients were done according to Lublin and Reingold[3].

Nine HC (6 females/3 males; mean age: 32.0 years [6.0]) were also included in the study.

An additional 3 RRMS-R patients (all females, mean age: 29.3 years [6.1]) were recruited from the Danish MS Center in Copenhagen for fresh isolation of peripheral blood to be utilized in suppression assays. Patients' blood was collected 36h after IFN-β injection, subjected to $FoxA1^{+}T_{reg}$ isolation and analysis of suppressive function. These experiments were approved by The Scientific Ethics Committee for Copenhagen and Frederiksberg (protocol KF01-041/95) and conducted after patients' consent. Additionally buffy coats from 14 healthy individuals were included for functional studies.

Blood sampling

Peripheral blood was collected by standard venipuncture into vacuum tubes with EDTA. PBMC were isolated by Ficoll-Isopaque density gradient centrifugation (Gibco-BRL) and stored in liquid nitrogen until used. PBMC were collected at baseline and after 24 months of IFN-β treatment. In HC, longitudinal PBMC taken at two time points separated by12 months (n = 2) and 24 months (n = 7) were used for comparison purposes. The study was approved by the Hospital Universitari Vall d´Hebron Ethics Committee [PR(AG)32/2008].

For functional studies, peripheral blood of IFN-β treated RRMS-R was collected by standard venipuncture into vacuum tubes with EDTA. PBMC were isolated by Ficoll-Isopaque density gradient centrifugation (Gibco-BRL), $FoxA1^{+}T_{reg}$ cells were purified using FACSAria and utilized in functional assays (described below).

Human blood lymphocytes preparation

Blood donors' buffy coats or MS patients' blood (10-12 ml) were used for preparation of lymphocytes. Ficoll-Paque PLUS (7.5 ml) (GE Healthcare, Cat. 17-1440-02) was utilized to carefully layer the diluted blood sample (15 ml). Cells were then centrifuged at 400 x g for 30–40 min at 18–20°C. Lymphocyte layer was collected and washed 3 times using PBS and centrifuged at 300 x g for 10 min.

Quantitative real time (RT)-PCR

Total RNA was isolated using a QIAGEN kit (QIAGEN), reverse transcribed into cDNA, and amplified and quantified by SYBR Green (Bio-Rad) detection. Relative mRNA expression was calculated using *Gapdh* gene expression as an endogenous reference. The primers for *Pd-l1* were (upper: 5'-CGC CCT TTT TAT TTA ATG TAT GGA-3'; lower: 5'-AAG TGA GGC GTC TGT GTT TGA G-3'). The primers for FoxA1 were as following:

3'UTR-1: 5'	CCTATTCTTGGCTCATCC
3'UTR-1: 3'	TGAAACCCGTTTGTCTAA
3'UTR-2: 5'	GACGTCCGAGATCTGAATGC
3'UTR-2: 3'	AAACCGGTTATGCACATTGG
3'UTR-3: 5'	TCCTGCATAAAGGCAGCACT
3'UTR-3: 3'	CCACCACACGAGTTTACAGG
ORF-2: 5'	TGTTAGGGACTGTGAAGATG
ORF-2: 3'	GTGTTCATGGAGTTCATAGAG

ORF-1: *Foxa1* (PPM04764H) were purchased from SAbiosiences (Qiagen)

FACS staining and sorting using FACSAria

After washing in FACS buffer (2% FCS in PBS), cells were incubated with anti-Fc receptor Ab (24.G.2, our hybridoma collection) at 10 μg/ml. Thereafter, cells were incubated with biotinylated, FITC- or PE-labeled Abs. For intracellular staining, cells were fixed and permeabilized using BD Cytofix/Cytoperm or using fixation and permeabilization solutions from Human Treg Flow Kit. All Abs were used at 1–5 μg/ml and were allowed to bind for 20 min on ice. Abs (1:200 dilution, otherwise indicated) were: FITC anti-human CD4 (BD, Cat. 555348), FITC anti-human TCRαβ (BD, Cat. 555547), PE anti-human TCRαβ (BD, Cat. 555548), APC-anti-human CD4 (eBioscience, Cat. 17-0049-41), PE anti-human CD47 (BD, Cat. 556046), FITC- PE-

Cy7 anti-human CD274/PD-L1 (BD, Cat. 558017), PE-anti-human PD-1 (eBioscience, Cat. 12-9969-42), Percp/cy5.5 anti-human CD69 (Biolegend, Cat. 310925), Bio-anti-human HLA-DR (Biolegend, Cat. 307614), APC-anti-human IL-10 (JES3-19F1, Biolegend), Alexa Fluor 488-anti-human IL-12/IL-23 p40/70 (eBioscience, Cat. 53-7129-41), anti-cleaved caspase 3 (Cell Signaling Technology, Cat. 9661, 1:1600), anti-phosphorylated AKT (Cell Signaling Technology, Cat. 4060L, 1:200), anti-phospho-p38 (Cell Signaling Technology, Cat. 9211, 1:400), Human Treg Flow Kit (FoxP3 Alexa Fluor 488/CD4 PE-Cy5/CD25 PE) (Biolegend, Cat. 320401), mouse anti-human (mouse) FoxA1 antibody (2F83, Abcam, Cat. ab40868) and secondary Alexa Fluor 488 goat anti-mouse (Invitrogen, Cat. A-11029) or Alexa Fluor 647 goat anti-mouse (Invitrogen, Cat. 558865), isotype control for FoxA1: purified mouse lgG1κ isotype (MOPC-21), anti-human IL-27/IL-35 EBI3 subunit-Alexa Flour 488 (R&D systems, Cat. IC6456G) and mouse lgG1 isotype control-Alex Fluor 488 (R&D systems, Cat. IC002G). APC or FITC-anti-mouse CD4 (L3T4), FITC or biotin-anti-mouse TCR (553169, H57-597), FITC-anti-mouse CD25 (7D4), isotype control for CD25: FITC-rat lgMκ isotype (553942, R4-22), PE-anti-mouse Pd-l1 (MIH5), isotype control for Pd-l1: PE-rat lgG2a isotype control (eBR2a, 8012-4321-025, eBioscience), FITC- or PE-anti-mouse FoxP3 (FJK-165, eBioscience), isotype controls for FoxP3: PE-rat lgG2a isotype control (eBR2a, 8012-4321-025, eBioscience) or FITC-rat lgG2a isotype control (eBR2a, 8011-4321-025, eBioscience), PE-anti-Ki-67 (B56), APC-anti- mouse CD69 (H1.2F3, eBioscience), FITC-anti-mouse CD47 (MIAP301). Violet-fluorescent reactive dye (Cat. L34955, Invitrogen) was used for LIVE-DEAD marker.

Cells were acquired with an FACSAria (BD Biosciences) using the FACSDiva software for acquisition after exclusion of duplets. Dead cells were discriminated in all staining using the LIVE/DEAD Fixable Dead Cell Stain Kit for 405 nm excitation (L34955, Invitrogen). FlowJo 8.8.6 (Tree Star) was used for further analysis.

For FACSAria sorting, T cells were stained with anti-CD4, anti-TCR and anti-PD-L1 Abs for 20 min at 4°C in the dark. FoxA1^{+}T$_{reg}$ cells were purified gated on TCR^{+}CD4^{+}PD-L1^{high} T cells with a FACSAria sorting program and exclusion of duplets.

FoxA1^{+}T$_{reg}$-cells sorting from RRMS-R and in vitro IFN-β-induced FoxA1^{+}T$_{reg}$

Lymphocytes were prepared freshly from PBMC and then cultured with 1,000 U/ml of human recombinant IFN-β (PBL InterferonSource) for 72 h. For FoxA1^{+}T$_{reg}$-cells sorting directly from RRMS patients PBMC or from *in vitro* IFN-b-induced, lymphocytes were first enriched with CD4^{+} T Cell Isolation Kit II (Miltenyi Biotec, Cat. 130-091-155) by automacs, then stained with anti-CD4, anti-TCR and anti-PD-L1 Abs for 20 min at 4°C in the dark. FoxA1^{+}T$_{reg}$ cells (TCRαβ^{+}CD4^{+}PD-L1^{high}) were purified utilizing a FACSAria sorting program.

CFSE labeling

To assay T-cell proliferation, cells were labeled with CFSE (Molecular Probes) by washing activated T-cell lines twice in staining buffer (PBS supplemented with 0.1% BSA) and re-suspending in 1 ml staining buffer. Freshly diluted 5 μM CFSE was added and cells were incubated in a 37°C water bath for 10 min with frequent mixing. An equal volume of FCS was added to stop labeling and cells were washed three times with culture medium containing 10% FCS.

In vitro suppression assay

To assay suppressive properties of FoxA1^{+}T$_{reg}$ cells obtained either from IFN-β (100U/ml) treated CD4^{+} T cells (for 48 h), or after 4 days transfected with pcDNA3.1FoxA1. Responder CD4^{+} T cells were MACS sorted with CD4^{+} T Cell Isolation Kit II (Miltenyi Biotec, 130-095-248), then labeled with CFSE and stimulated with plate-bound anti-CD3 (when indicated also solube anti-CD28 is used) for 24 h. FoxA1^{+}T$_{reg}$ cells were purified by FACSAria and labeled with Texas-red tracker (Genovis) (for some experiments). Suppressor and responder cells were co-cultured in a

new culture plate without anti-CD3 at a 1:1 (suppressor : responder) ratio. After 24 h, cells were stained with violet dead cell marker (Invitrogen) or 7AAD and analyzed by FACSAria.

For studying the FoxA1's role on suppressive function of $FoxA1^{+}T_{reg}$ cells, purified $CD4^{+}$ T cells were labeled with CFSE, then treated with anti-CD3 (145-2C1, 1μg/ml) and anti-CD28 (37.51, 10μg/ml) for 24 hours, served as responder T cells. Purified $CD4^{+}$ T cells were tranfected with FoxA1 siRNA or control siRNA or were purified from WT versus *Ifnar*$^{-/-}$ mice and used as suppressor cells. Suppressor and responder T cells were co-cultured in a new culture plate without anti-CD3 at a 1:1 (suppressor : responder) ratio. After 24 h, responder cell's proliferation was analysed by FACS.

For IL-2 effect on suppressive function of $FoxA1^{+}T_{reg}$ cells, 2.4 pg/ml of recombinant IL-2 was added to the co-culture. For involvement of Pd-l1 in suppressive function of $FoxA1^{+}T_{reg}$ cells, neutralizing Abs were anti-mouse Pd-1 (RMPI-14, Biolegend), anti-mouse Pd-l1 (MIH5, eBioscience), anti-mouse B7.1 (IG10, Pharmingen) and anti-mouse B7.2 (2D10, Pharmingen). For involvement of caspases and Pd-l1 in suppressive function of $FoxA1^{+}T_{reg}$ cells, caspase inhibitor Z-VAD-FMK (G7231, Promega, USA) and anti-mouse Pd-l1 (MIH5, eBioscience) or anti-human PD-L1 (329702, Biolegend) were used.

Foxa1 silencing of primary moues T cells by a siRNA approach

Accell SMART pool small-interfering RNA (siRNA) was purchased from Dharmacon (cat:E-046238-00, Thermo Scientific), and was introduced into cells according to manufacturer's protocol. Briefly, SMART pool siRNA combines four different siRNAs to reduce off-target effects. The Accell siRNA is also designed for optimal delivery to hard-to-transfect cells and no transfection reagents are required to introduce the siRNAs. For Foxa1 Accell SMART pool contained 4 siRNAs all targeting 3'UTR region. These siRNAs were introduced separately or in pool to purified mouse $CD4^{+}$ T cells in the Accell delivery medium (cat:B-005000-100, Thermo Scientific) and incubated for 72 h prior to suppressive assays. Delivery efficiency and siRNA specificity were examined by

qPCR and intracellular staining of FoxA1. Accell non-targeting control siRNA (cat:D-001910-01-05, Thermo Scientific) as a control. Sequences of 4 siRNAs all targeting 3'UTR region 1:CGGACGUCCUUAAGUGAAA, 2: CUAUGGACUUAAUAUCAUG, 3: GGCUCAUCCAGUGUUAAUG, 4: GACUGUUACUUUAUUAUUG

In vivo suppression assay in chimeric NOG mice

Purified OT-II $CD4^+$ T cells were labeled with CFSE, then treated with anti-CD3 (145-2C1, 1μg/ml) and anti-CD28 (37.51, 10μg/ml) for 24 hours, served as responder cells. Suppressor cells were obtained from tranfected FoxA1 siRNA or control siRNA in purified OT-II $CD4^+$ T cells. For some experiments, $CD4^+$ T cells were purified from *ifnar*$^{-/-}$ and WT mice as suppressor cells. NOG mice received 100 μl of $1X10^6$ with pre-activated responder cells *i.v.*, after 24 hours $1X10^6$ of suppressor cells were transferred to each mouse. Simultanously indicated mice were treated *in vivo* with mouse recombinatant IFN-β (100 U). After an additonal 24 hours, NOG mice were terminated and spleens were taken for further analysis.

BrdU and PI cell cycle staining

Cells were labeled with BrdU (PerkinElmer, Cat. AD0200-A) at 10 μM for 1 h, cells were harvested and single-cell suspensions prepared in buffer (PBS + 0.1% BSA), washed twice and resuspended at $1x10^6$ cells/ml. Cells were prepared in 1-ml aliquots in a 15-ml polypropylene, V-bottomed tube. 3 ml cold absolute ethanol was added. Cells were fixed for at least 1 h on ice and then resuspended in 2 M HCl at room temperature for 30 min. 0.1 M sodium tetraborate (pH 8.5) was added for 2 min. After washing with PBS + 0.1% BSA + 0.2% Tween-20, 20 μl 1/10 dilution anti-BrdU Ab (BD, Cat. 556028) was added directly to the cell pellet and incubated for 20 min at room temperature in the dark. Cells were washed and 1 ml of PI/RNase Staining Buffer (BD

Biosciences, Cat. #550825) was added to the cell pellet and thoroughly mixed before analysis by FACS.

APC preparation and co-culture

For APCs sorting, $CD3^+$ T Cell Isolation Kit (Miltenyi Biotec, Cat. 130-050-101) was used for automacs $CD3^+$ T Cells separation, and the negative population was used as APCs. APCs were treated with LPS (100ng/ml) for 48h prior to co-culture with FACSAria purified $FoxA1^+T_{reg}$ and control T cells ($CD4^+Pdl1^{lo}$) at ratio of 1:1 for additional 24h. Supernatants were collected for ELISA and cells were stained and analyzed with FACS.

ELISA

Nunc Maxisorp plates (Nunc) were coated with primary antibodies at 4°C overnight. IL-12 (p70) (cat:BMS238), IL-17A/F (cat:88-7117-88) ELISA kits were purchased from eBiosience. ELISA was performed as recommended by manufacturer.

Plasmids

Foxa1 was synthesized by Geneart into pMA with 5' Hind III and 3' Not I sites. *Foxa1* was transferred to the mammalian expression vector pCDNA3.1 (Invitrogen) by cutting the pMAFoxA1 and pCDNA3.1 HindIII and NotI followed by ligation of the pCDNA3.1 or *FoxA1* HindIII/NotI cut fragments. Plasmid was prepared with a maxiprep kit from Macherey Nagel.

PD-1 silencing of primary human T cells by a siRNA approach

5-10x10^6 purified $CD4^+$T cells from healthy donors were transfected with 100 nM of a PD-1 siRNA (Predesigned siRNA directed against human PDCD1_3 FlexiTube siRNA (5 nmol) Cat. No./ID: SI00071323, QIAGEN, sequence; CCCTGTGGTTCTATTATATTA) or Universal Negative Control 1/UNC siRNA (SIC001, Sigma-aldrich) using the Amaxa Human T cells Nucleofector Kit

(VPA-1002) (program U-014) according to the manufacturer's instructions.

Amaxa gene transfection

$1x10^6$ purified $CD4^+$ T cells from C57BL/6 mice were transfected with 4 μg of a pcDNA3.1FoxA1 or a control plasmid pcDNA3.1 using the Amaxa mouse T cells Nucleofector Kit (DPA-1007) (program X-001) according to the manufacturer's instructions. The transfection efficiency was evaluated after 4 days of transfection by qPCR, using different primers distinguishing 3'UTR region versus ORF, FoxA1 staining and FACS analysis.

Western Blot

Proteins were extracted in 40 μL SDS loading buffer (Invitrogen, USA) from pcDNA3.1foxa1 transfected $FoxA1^+T_{reg}$ cells and pcDNA3.1 transfected control T cells. 30 μL of protein lysate was loaded on 4%-12% SDS-PAGE gels and proteins were blotted onto Hybond-C extra nitrocellulose membranes (GE Healthcare, UK) membranes. The membranes were blocked in 5% milk in PSB-Tween-20 (0.05%) 1 hr RT and incubated with: Rat anti-FoxP3 (FJK-16s, eBiosience 1:2000), goat anti-FoxA1 (ab5089, Abcam 1:1000), rabbit anti–pc-Fos (D82C12, Cell Signaling Technology 1:1000), rabbit anti–c-Fos (9F6, Cell Signaling Technology, 1:1000), rabbit anti-b-actin (13E5, Cell Signaling Technology 1.3000) 1 hr RT and then with HRP-conjugated secondary antibodies. The blots were developed using the ECL technique (Millipore, MA, USA).

Immuno/histochemistry

Brains and Spinal cords of mice with EAE were dissected and immediately embedded in OTC compound (Sakura Finetek Denmark ApS, Værløse, Denmark) and snap-frozen in isopentane on dry ice. Tissues were cryo-sectioned in 10 μm slices and kept at −80 °C until staining. Tissue sections were fixed in 4% Paraformaldehyd (PFA) for 10 minutes, either stained with Hematoxylin

and Eosin (H&E) staining or different antibodies were used for staining and visualized by DAB as described[4]. Slides were visualized under light microscopy.

Immunoflourescent cytochemistry

FoxA1^{+}T$_{reg}$ and control cells (Pd-l1^{low} T cells) were generated utilizing purified CD4^{+} T cells treated with mouse recombinant IFN-β (100U/ml) for 48h or amaxa transfection of with FoxA1, then sorted with FACSaria and applied to slides, fixed with 4% PFA and subsequently stained. Alexa Fluro® 568–labeled anti-TCRβ (H57-597, BD Biosciences) and biotinylated anti-PD-L1 (MIH5, eBioscience) were used, followed by Streptavidin and Alexa Fluro® 488 conjugated anti-rat IgG. In addition, Antibodies were used as following: mouse FoxA1-specific antibody (Abcam, ab40868,1:500) and secondary Alexa Fluor 568 goat anti-mouse (A-11004, Invitrogen,1:300) and rabbit anti–pc-Fos (5348, Cell Signaling Technology, 1:800) followed by Alexa Fluor 488–labeled secondary antibody (A-11029, Invitrogen, ,1:300); biotin anti-mouse Pd-l1 (MIH5, eBiosience, 1:200), followed by secondary streptavidin-Cy3 (S7973-89J, US biological, 1:300); and biotin hamster anti–TCR-b (H57-597, BD Biosciences, 1:200) and followed by Alexa Fluor® 568 Goat Anti-Hamster IgG (A-21112, Invitrogen, 1:300). DAPI (D3571, Invitrogen) was used for nuclei staining.

For some experiments, pcDNA3.1FoxA1 transfected FoxA1^{+}T$_{reg}$ cells and pcDNA3.1 transfected control T cells were used. All staining was visualized under a Zeiss fluorescence microscope.

Affymetrix data analysis

Preparation and analysis: We prepared RNA for Affymetrix analysis in biological triplicates from three independent experiments, from CD4^{+} (EncT cells), CD4^{hi}PD-L1^{hi} (nFoxA1^{+}T$_{reg}$ cells), or CD4^{+} membrane-bound TGF-β^{+} (T$_{reg}$ cells) or IFN-β-induced / iFoxA1^{+}T$_{reg}$ cells/CD4^{+}Pd-l1^{hi}

(purified murine $CD4^+$ T cells were treated with 100U/ml of murine IFN-β for 48 h). We purified T cells ($FoxA1^+T_{reg}$ cells and T_{reg} cells) by FACSAria, extracted RNA by Trizol (Sigma, followed by DNAseI (Invitrogen) digestion, and a subsequent Trizol RNA purification. RNA purity was examined by spectrophotometry and Affymetrix analysis (SCIBLU, Affymetrix core laboratory, Lund University) was performed. Affymetrix data files were analyzed with the Arraystar 2 software (DNASTAR). Data sets were quantile normalized and processed by the PLIER (Affymetrix) algorithm set to subtract mismatch probes from perfect match probes (PM-MM). Intensity values were $\log_2$-transformed to obtain normal-distributed data for an unpaired, two-tailed Student's *t*-test assuming equal variance, set to filter for a differential regulation confidence of 95% ($P \leq 0.05$). Signal intensity ratios that differed by 1.5-fold between triplicate averages were selected as differentially expressed genes. The microarray data generated in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number; GSE54490 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54490).

Venn diagrams from the normalized PLIER analyzed data were created with oneChannelGUI (www.bioconductor.org). Assessment graphs of hybridization quality are available upon request.

We also analyzed the affymetrix data by the MAS5 algorithm according to manufacturer's instructions. Probe sets with were filtered for apoptotic GO annotations and for MAS5 present calls.

Comparison between gene profile of $nFoxA1^+T_{reg}$ and published Exhausted T cells: For gene specific in $nFoxA1^+T_{reg}$ cells in comparison with exhausted T cells, microarray CEL file for published exhausted T cells[5] was used (GSE9650). Venn diagrams and gene overlapping were analyzed by a statistics program R. Additional analyses were performed using analogous approach to compare $nFoxA1^+T_{reg}$ cells with published exhausted CD8/CD4 T cells data sets[6,7]. Overlapping genes were only 6 (data not shown).

Comparison between gene profile of $nFoxA1^+T_{reg}$ and published natural and induced T_{reg} cells: $nFoxA1^+T_{reg}$ affymetrix data (Mouse Genome 430 2) generated here, as well as published T_{reg}

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affymetrix data[8,9,10](GSE7460, GSE14415, GSE40685) were quantile normalized and summarized for each comparison using justPlier implementation of Plier algorithm in R. To draw heatmaps, heatmap2 function from gplots package in R was used. Gene Set Enrichment Analysis (GSEA) was performed using pre-processed data fed into Java implementation of GSEA (v 2.0.12, Broad Institute) and analyzed with default settings (except gene set permutation) using gene sets from MSigDb (v 3.1).

Transcription factors were called among the differentially expressed genes by using Gene Ontology term GO:0003700 "sequence-specific DNA binding transcription factor activity". Overlap with putative T_{reg} regulating transcription factors[11], and visualization were performed in R.

Identifying common gene profile for $FoxA1^{+}T_{reg}$ cells: For identifying common regulated genes from IFNβ-induced $FoxA1^{+}T_{reg}$ ($iFoxA1^{+}T_{reg}$) and neuron-induced $FoxA1^{+}T_{reg}$ ($nFoxA1^{+}T_{reg}$) cells, these CEL files and encephalitogenic T cells (EncT) CEL file were quantile normalized and summarized together using justPlier function in R. Probe sets were filtered for 1.5 fold change and p-value £ 0.05 (moderated t-statistic no multiple testing correction). Overlap between the two differentially regulated probe sets of $FoxA1^{+}T_{reg}$ cells, under condition that direction of regulation in $iFoxA1^{+}T_{reg}$ and $nFoxA1^{+}T_{reg}$ vs. EncT should be the same (936 commonly regulated probe sets) with respective expression values from each comparison were separately fed into GSEA program (Broad Institute). Gene set Common Pathways (v.3.1) was analyzed, with default setting, except the permutation was done by gene sets and minimum gene set size cut-off was reduced to 3. Top 20 gene sets were tabulated and compared, to find the overlapped gene sets known in biological pathways. Gene names from these gene sets (core enriched and not enriched) were extracted, mapped back to 936 commonly regulated probe sets (except one, probably due to different versions of Affymetrix annotation databases in GSEA and R) and a heatmap of expression values was plotted.

Venn diagram of overlap between differentially regulated probe sets that are regulated in the same direction in $iFoxA1^{+}T_{reg}$ and $nFoxA1^{+}T_{reg}$ vs. EncT cells were made. Shared regulated genes

by iFoxA1^{+}T$_{reg}$ and nFoxA1^{+}T$_{reg}$ vs. EncT cells that are involved in top 20 commonly regulated pathways identified by GSEA were utilized to made the heatmap of FoxA1^{+}T$_{reg}$ gene profile. Some genes are present more than once, either due to mapping to two commonly regulated probe sets or due to presence in two different pathways.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed essentially as described[12] with the following modifications. Sonications were performed on a Biruptor Next Gen (Diagenode) set for 30s on, 30s off for 12 cycles. Goat IgG purified by protein G sepharose from goat serum (Sigma) was used as a negative control for the FoxA1 antibody (Abcam, ab5089). 4 μg of each antibody was used. The ChIPed DNA was purified on QIAquick PCR purification kit (Qiagen cat no 28104) and qPCR was performed with primers from table 1 with the Lightcycle 480 DNA SYBR Green I Master Mix (Roche). Primers for the selected sequences were designed using Primer3 (v. 0.4.0), see below table.

qPCR primers for putative FoxA1 binding sites and control.

Pdl1-A 5' (PF5')	ATTAAGGGGGCATGGACAAC
Pdl1-A 3' (PF3')	CAGCACAAGCTGGACTTCAA
Pdl1-B 5' (PF5')	TGCAGGTAAGGGAGCATCTT
Pdl1-B 3' (PF3')	CTTTAAAGTGCCCTGCAAGC
Gapdh 5' (G3')	AGTGCCAGCCTCGTCCCGTAGACAAAATG
Gapdh 3' (G5')	AAGTGGGCCCCGGCCTTCTCCAT

Electro mobility shift assay (EMSA)

To purify nuclear FoxA1, we transduced 3T3L1 cells to constitutively overexpress FoxA1 (3T3-FoxA1) and the nuclear fraction was purified as previously described.

Mutated transthyretin (mTTR) is a FoxA1 binding oligo[13] with additional CAG nucleotides in the 3' end, which served as a positive control for FoxA1 binding. *Pdl1-A* was selected as a potential FoxA1 binding site upstream of *pdl1* gene using ContraV2[14], with a FoxA1 MA0148.1 binding sequence matrix from Jaspar database[15]. *Pdl1-B* contains a FoxA1 binding sequence in the promoter region of *pdl1* gene that has been identified based on FoxA1 ChIP-seq peak data identified in ZR751 breast cancer cell line [16]. The precise location of FoxA1 binding sequence was predicted using Clover[17] with MA0148.1 FoxA1 matrix. *Pdl1-A* and *Pdl1-B* EMSA probes sequence were selected from genomic mm9 assembly (UCSC genome browser) (see Table below) and are within the amplicon of the *Pdl1-A* and *Pdl1-B* primer pairs used in the ChIP assay.

Oligos were labeled with ^{32}P-ATP (PerkinElmer, cat. no. BLU002Z) using T4 polynucleotide kinase (New England Biolabs, cat. no. M0201) following manufacturer's protocol. 5%-Tris-borate EDTA/ polyacrylamide gels were used for EMSA as described previously[18], with 2ul nuclear extracts and where applicable 1ul of antibody for super shift. All chemicals were from Sigma unless otherwise stated. FoxA1 antibodies; clone 2F83 (05-1466, Millipore, 1 μg) used to verify the specificity of results and ab5089 (Abcam, 1 μg) that was used in super shift assays.

EMSA oligos.

mTtr	GTCTGCTAAGTCAATAATCAGAATCAG
mTtr	CTGATTCTGATTATTGACTTAGCAGAC
Pdl1-A	TCGGAGGAGTAGAAACAGGTGGAGGAG
Pdl1-A	CTCCTCCACCTGTTTCTACTCCTCCGA
Pdl1-B	GTGTTTGTTTGTTTTTGTTTTTATCGA
Pdl1-B	TCGATAAAAACAAAAACAAACAAACAC

Luciferase assay

13000 mycoplasma tested, low passage 3T3L1 (ATCC CL-173) cells were reverse transfected with lipofectamine 2000 (Invitrogen) in 96 well plates according to the manufacturers

recommendations with 10ng pRLCMV (Promega), 95ng pGL3 (Promega) and pCDNA3.1 (Invitrogen) or pCDNA3.1FoxA1 mammalian expression plasmids. Media was removed and 50ul PBS was added to each well, next luciferase activity was measured according to the Dual glow luciferase protocol with 50 ul Luciferase and Renilla substrate (Promega). Data was normalized to the activity of a pRLCMV internal control plasmid.

Plasmid construction

A reporter for the activity of the mouse *Pdl1* promoter was constructed by amplification of a 1-2499 bp large fragment directly upstream of the PD-L1 gene (mm9, chr19:29439695+29442193).

The primers 5'KpnI 5'-GGTACCTTCTGGAGGATACCAAAACTTCA-3' and 3'MluI 5'-ACGCGTAGTCGCGCTAGGACCAATTA-3' were used for the PCR and the amplified PCR fragment was cloned into the pGL3 basic vector with standard technique (pGL3 *Pdl1*prom). Plasmid for transfection was purified with a Machinery nagel maxiprep kit.

The fold enrichment was calculated as: 1. $CT_{samples}$-CT_{input} = ΔCT. 2. Foxa1(ΔCT)-lgG (ΔCT)= ΔΔCT. 3. [2^-(ΔΔCT)] of pcDNA3.1foxa1/[2^-(ΔΔCT)] of pcDNA3.1control= fold enrichment.

Statistical evaluations

Statistical evaluation was performed using GraphPad Prism. For analyzing differences in clinical scores (including mean clinical score, mean maximal score), One-way ANOVA Kruskal-Wallis test with multiple comparisons and Mann-Whitney tests were used. Disease incidence, total recovery frequency, and relapse frequency were evaluated using the χ^2 test. For FACS analysis, cell proliferation and cell death assays, unpaired Student's *t*-test was used for two groups comparison. For multiple comparisons, one-way ANOVA with Newman Keuls test was used. For human patients comparison before and after treatment, paired Student's *t*-test was used. A value of $P < 0.05$ was considered significant. The assumption of normal distribution was investigated and data were

found to have a well–defined mean, which indicating the normal distribution tests can be used.

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