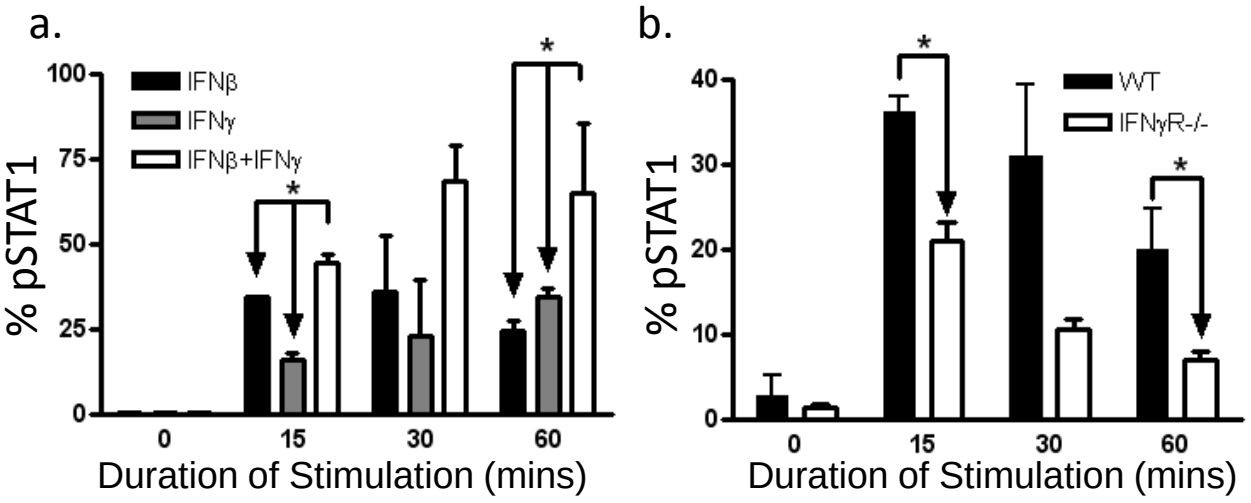

Supplementary Fig. 1: Synergy of IFN-β and IFN-γ on STAT1 activation. a) Spleen cells C57BL/6 were stimulated with IFN-β or IFN-γ or both for 0, 15, 30 and 60 mins and phosphorylation of STAT1 in CD4 T-cells was assessed by flow cytometry. b) IFN-β requires IFN-γ for optimal STAT1 signaling. C57BL/6 and IFNγR$^{-/-}$ spleen cells were stimulated with IFN-β 0, 15, 30 and 60 mins and phosphorylation of STAT1 in CD4 T-cells was assessed by flow cytometry. *p<0.05.

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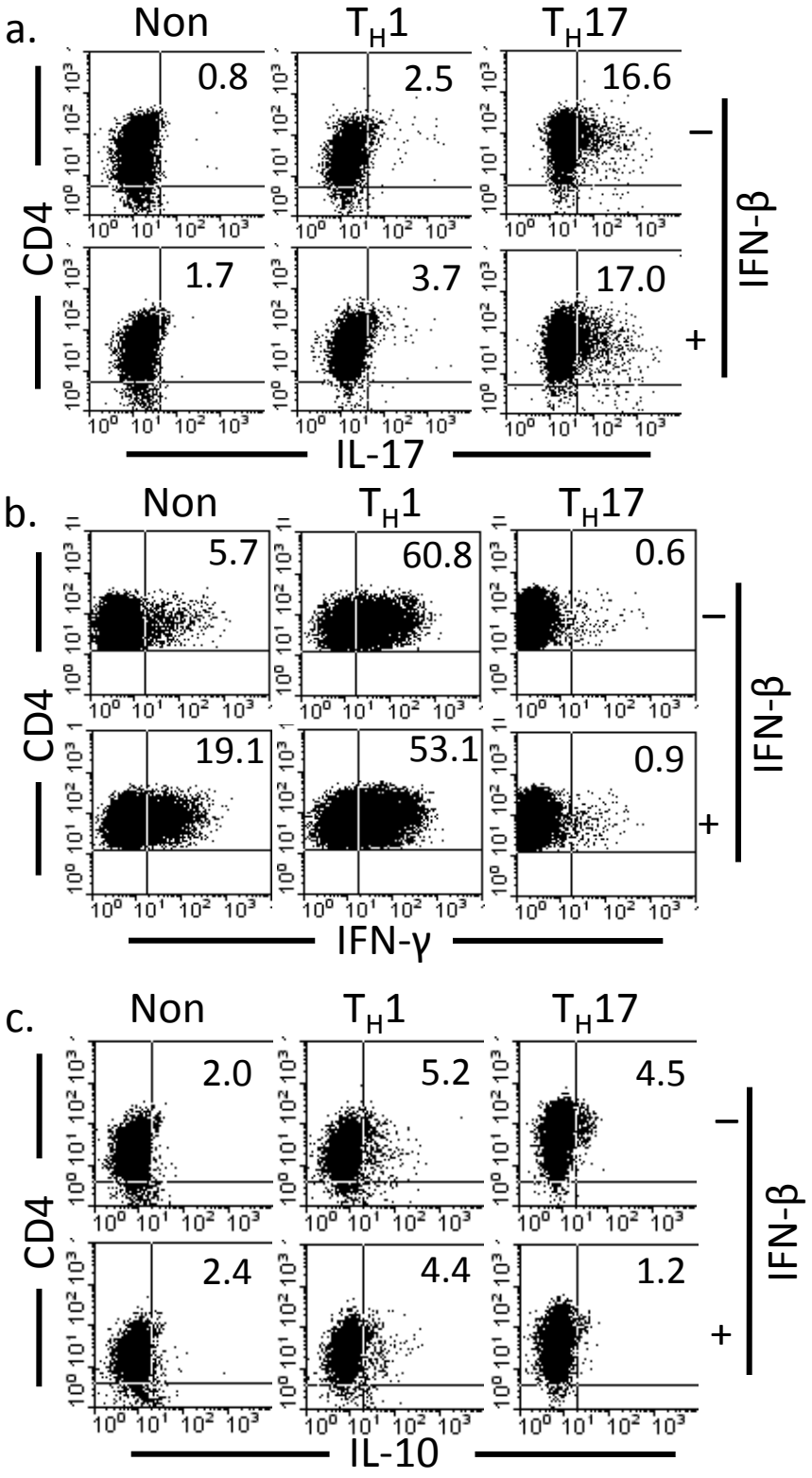

Supplementary Fig. 2: Direct effect of IFN-β on CD4 T-cells. Purified CD4 T-cells were stimulated with plate-bound anti-CD3 and anti-CD28 in non-polarizing, T_H1 and T_H17 conditions in the presence or absence of IFN-β. a) IL-17, b) IFN-γ and c) IL-10 production in CD4 cells were assessed by flow cytometry.

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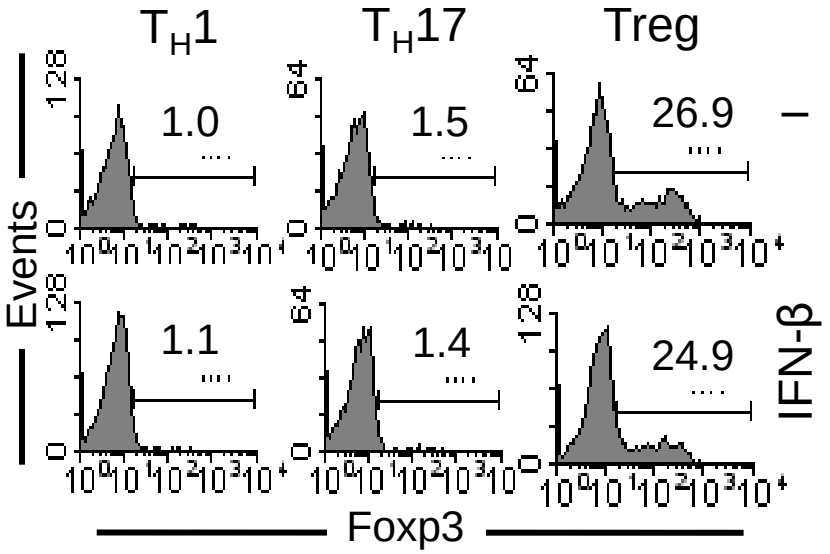

Supplementary Fig. 3: IFN-β does not affect the differentiation of Foxp3$^+$ Tregs. CD8 depleted spleen cells stimulated with or without IFN-β in TH1 (IL-12), TH17 (TGFβ/IL-6) and Treg (TGFβ) conditions and percentage of CD4$^+$ FoxP3$^+$ cells was assessed by flow cytometry.

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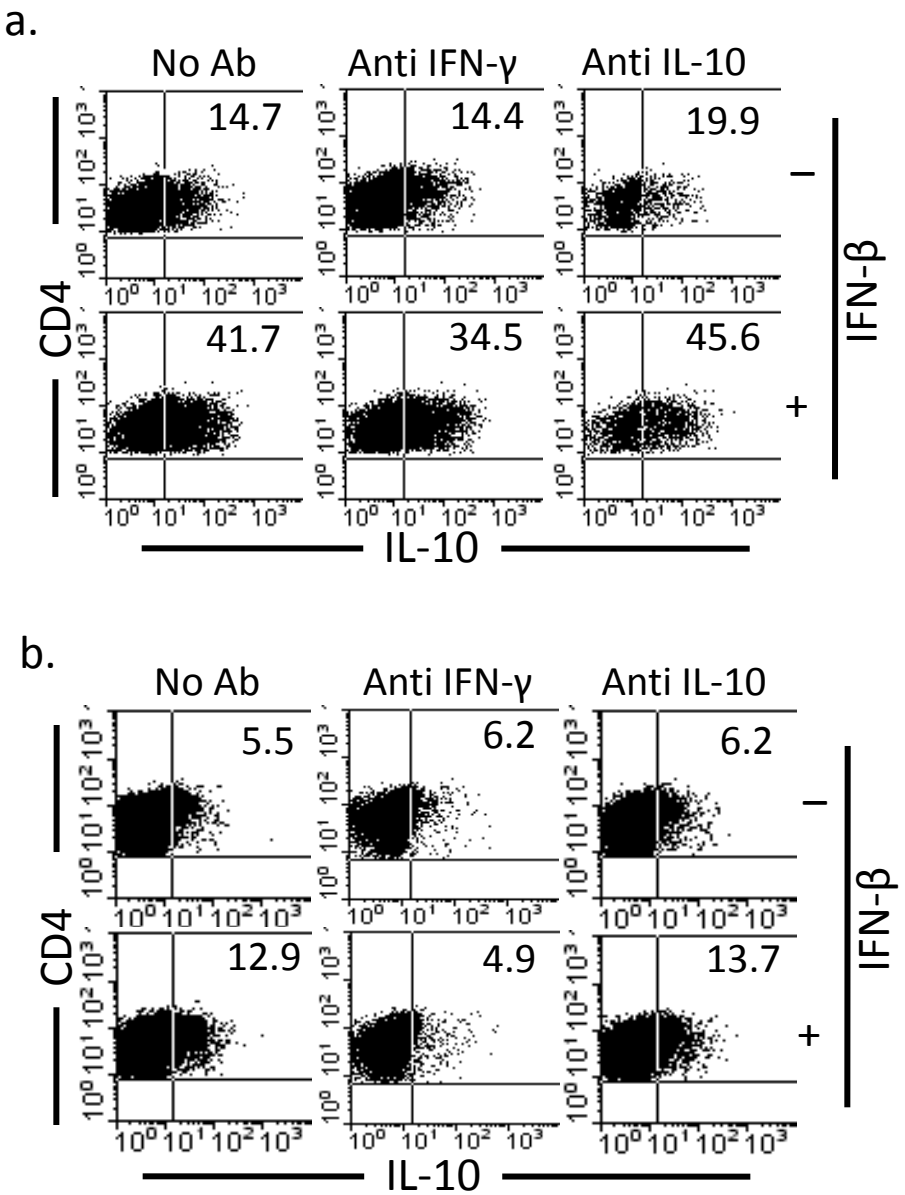
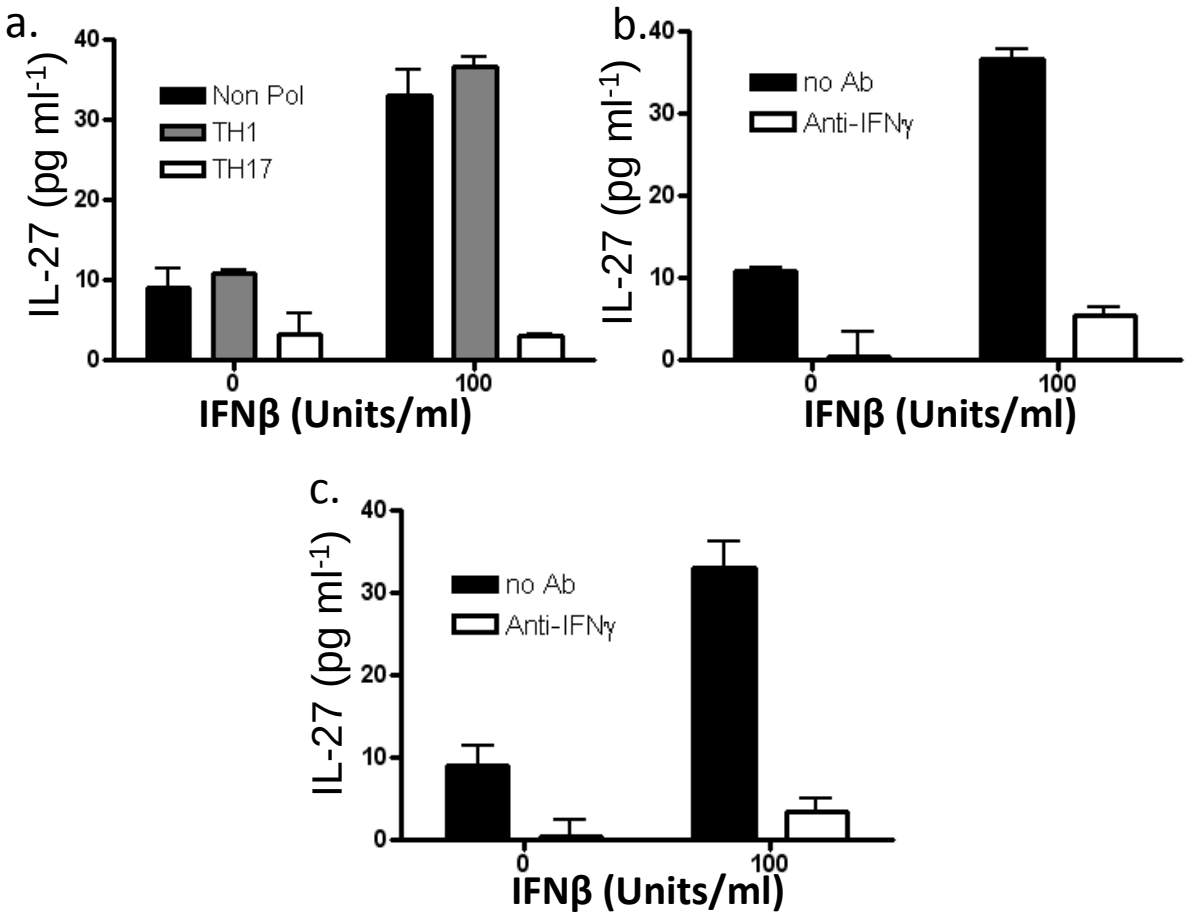


Supplementary Fig. 4: Effect of inhibiting IFN-γ or IL-10 signaling during the induction of IL-10 by IFN-β in a) T_H1 conditions and b) T_H17 conditions with APCs. CD8 depleted spleen cells were stimulated with or without IFN-β in T_H1 or T_H17 conditions in the presence or absence of anti-IFN-γ or anti-IL-10.



Supplementary Fig. 5: Effect of IFN-β on IL-27. a) IFN-β induces IL-27 in non-polarizing and T_H1 conditions but not T_H17 conditions. CD8 depleted spleen cells were stimulated with or without IFN-β in non-polarizing, T_H1, and T_H17 conditions and IL-27 was analyzed by ELISA. (b and c) IFN-β requires IFN-γ to induce IL-27 in non-polarizing conditions (b) and T_H1 conditions (c). CD8 depleted spleen cells were stimulated with or without IFN-β in non-polarizing, T_H1 conditions in the presence or absence of anti-IFN-γ and IL-27 was analyzed by ELISA. Results for these experiments are the mean ± SD of triplicates.

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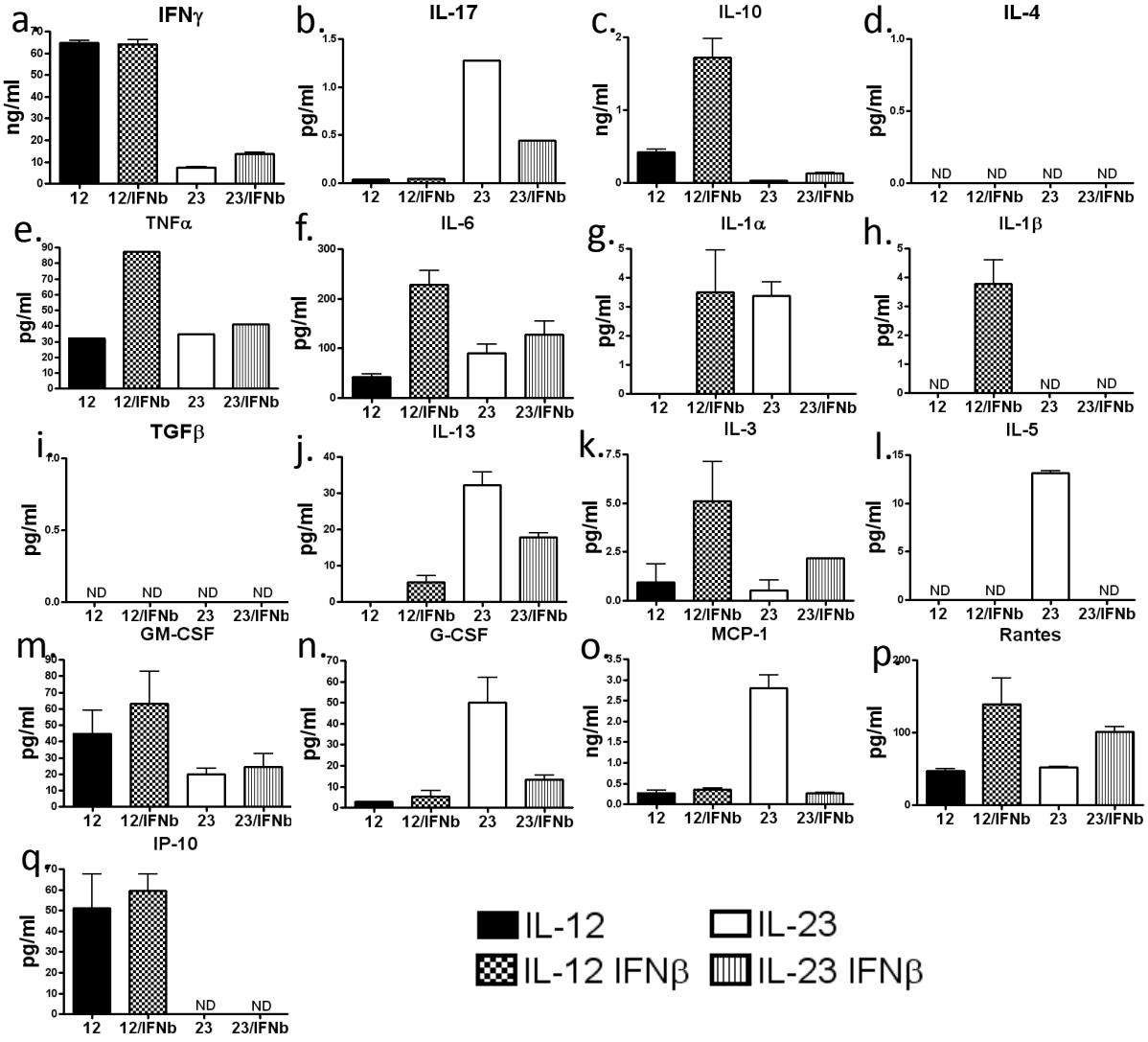

Supplementary Fig. 6: Effect of IFN-β on chemokine/cytokine profiles in antigen specific T_H1 and T_H17 differentiation. Lymph nodes from MOG_{p35-55} immunized mice were re-stimulated in MOG_{p35-55} for 3 days with IL-12 or IL-23 in the presence or absence of IFN-β. Chemokines and cytokines were assessed by Luminex multiplex analysis or ELISA.

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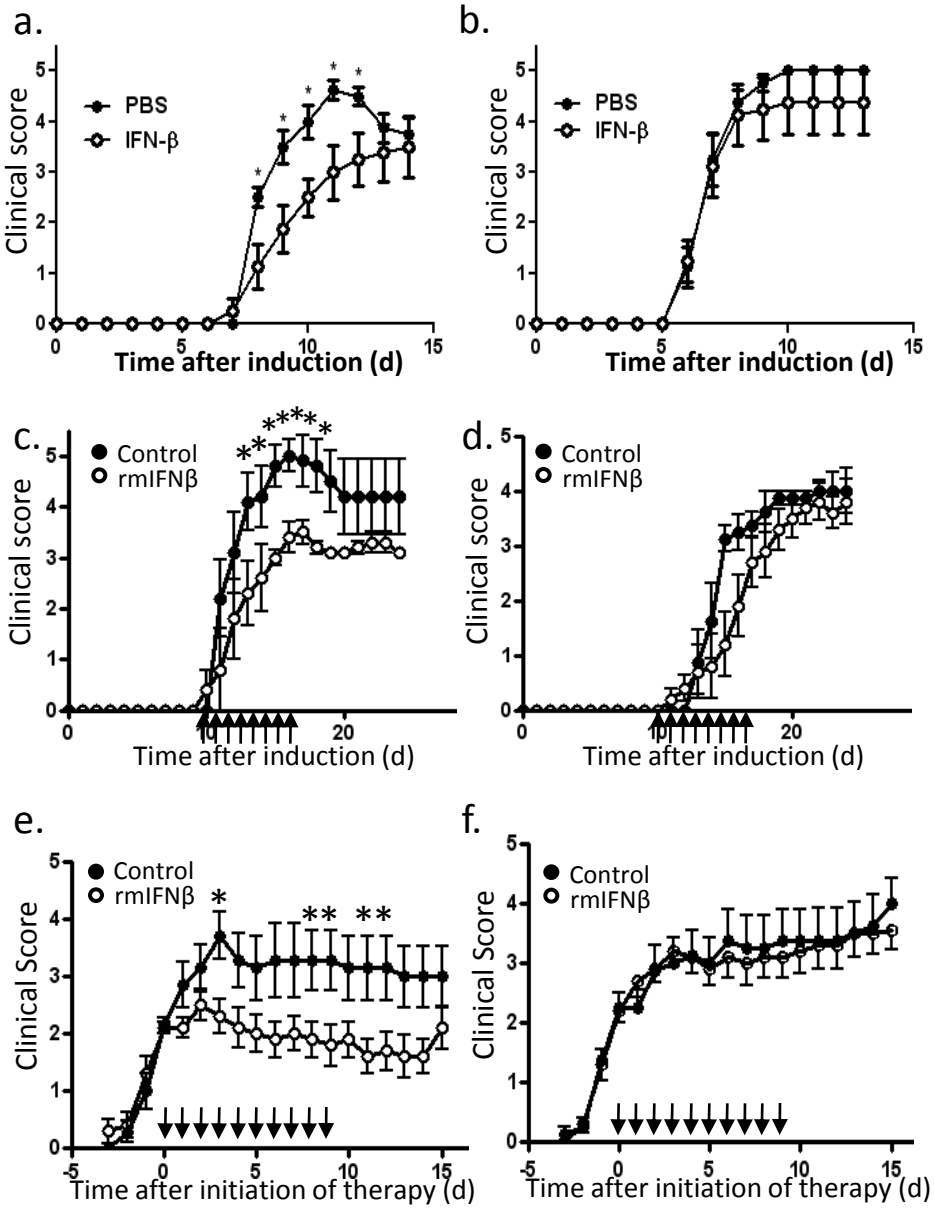

Supplementary Fig. 7: Effects of IFN-β treatment in different EAE models. (a and b) Clinical scores from SJL mice with passive EAE induced by adoptive transfer of (a) T_H1 and (b) T_H17 cells that were treated with rmIFN-β or PBS every second day from day 0 to 10 post transfer (*n*=6 mice per group). (c and d) Clinical scores from (c) C57BL/6 and (d) *IFNγR*$^{-/-}$ mice treated daily with rmIFN-β of PBS from day 10 to day 17 post EAE induction (*n*=4 to 5 mice per group). (e and f) Clinical scores from (e) C57BL/6 and (f) *IFNγR*$^{-/-}$ mice treated daily for 10 days with rmIFN-β or PBS beginning at disease score of 2 or 3 (*n*=6 to 9 mice per group). Treatment doses indicated with arrows. **p*<0.05.

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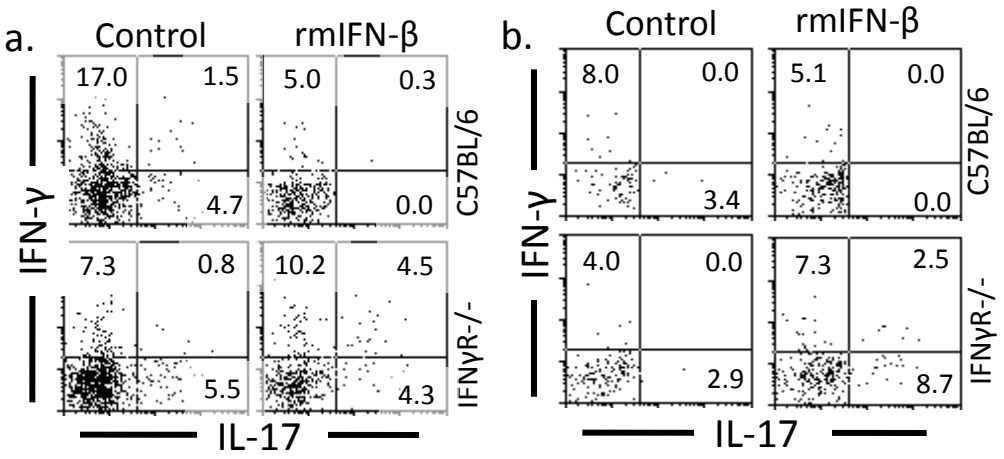

Supplementary Fig. 8: Frequencies of the IFN-γ and IL-17 producing CD4 T-cells in the spinal cords (a) brainstem/cerebellum (b) 12 days post induction of EAE in C57BL/6 or IFNγR$^{-/-}$ mice treated with rmIFN-β or PBS.

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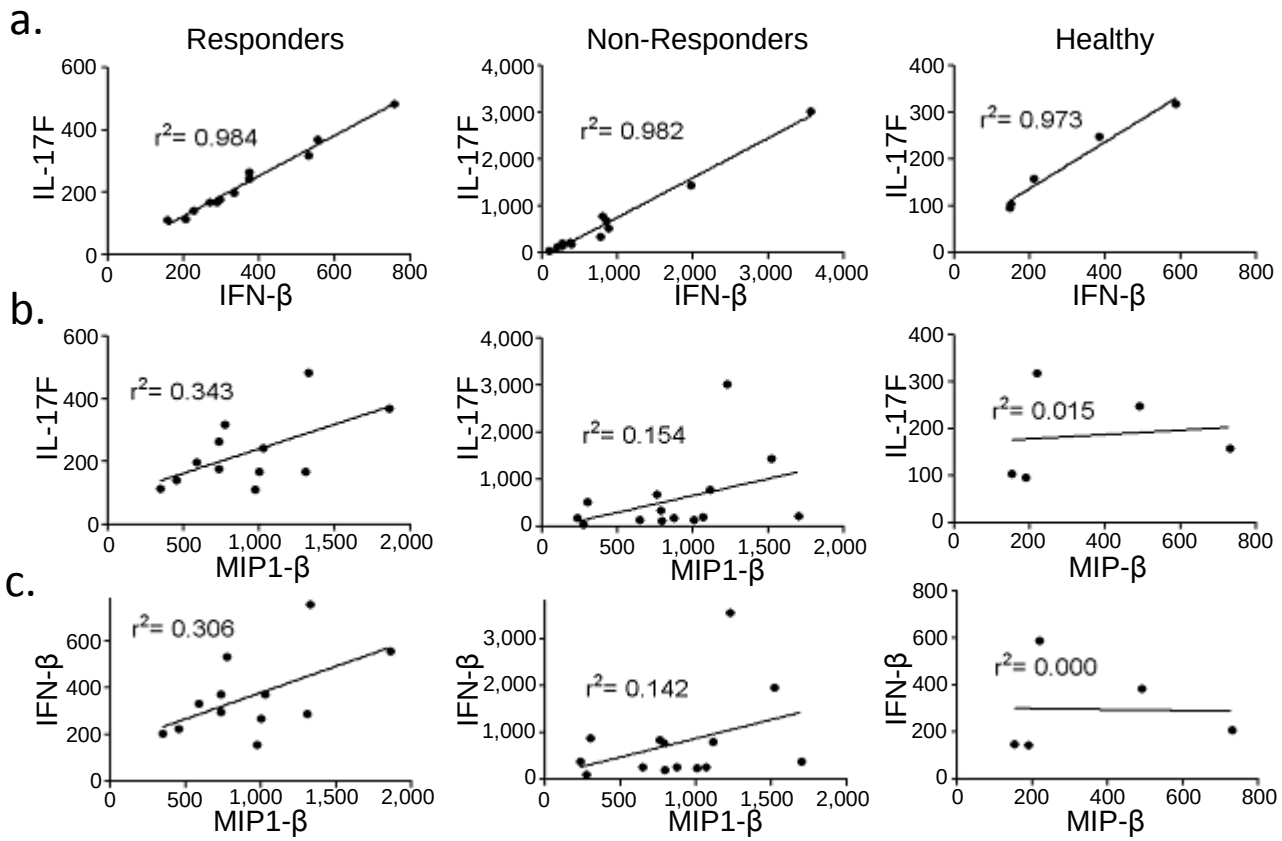

Supplementary Fig. 9: Correlation of a) IL-17F vs IFN-β levels, b) IL-17F vs MIP1β levels and c) IFN-β vs MIP1β in serum from responders, non-responders and healthy controls. R^2 values close to 1 demonstrate that the cytokines are positively correlated.

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Supplementary Table 1. Demographic and clinical characteristics of patients with relapsing remitting multiple sclerosis and their clinical response to IFN-β therapy.

	Responder	Non-responder
Number	12	14
Female/Male (n)	10/2	11/3
Median age at onset (yr)	27.6 [24.5; 35.8]	26.7 [19.3; 36.0]
Median age at start IFN-β (yr)	33.5 [30.3; 39.5]	33.0 [23.0; 37.8]
Median EDSS score around start IFN-β	2.5 [2.0; 3.5]	2.5 [1.8; 4.3]
Relapse rate in 2 yrs before start IFN-β	2 [2-3]	2 [1-3]
Relapse rate in 2 yrs after start IFN-β	0 [0; 0]	2 [1.5; 2.0]
Steroid interventions before start IFN-β (n)	0 [0; 2]	1 [0; 3]
Steroid interventions after start IFN-β (n)	0 [0; 0.5]	2 [1; 3]
Duration of IFN-β treatment (months)	80 [46; 141]	56 [38; 104]
Avonex	4	5
Rebif	2	8
Betaferon	6	1

Median values are shown with 25 and 75 percentiles.
IFN-β = Interferon-beta.
EDSS = Expanded Disability Status Scale

Supplementary Methods.

Mice. SJL, and Ifngr1$^{tm/Agt}$/J (*Ifngr1*$^{-/-}$) mice were purchased from Jackson Laboratory and C57BL/6 mice were purchased from Jackson Lab or NCI-Fredrick bred at Stanford and/or UAB. B6 *Stat1*$^{-/-}$ mice were provided by R. Lorenz (UAB). All animals were housed and treated in accordance to with institutional guidelines and approved by the IACUC.

EAE induction. Age and sex matched C57BL/6 and *Ifngr1*$^{-/-}$ mice were induced with EAE by an immunization 150 g of MOG p35–55 (Biosynthesis) emulsified in CFA followed by an intraperitoneal injection of with 500 ng of *Bordetella pertussis* toxin (Difco Laboratories) in PBS at the time of, and two days following immunization.

The typical clinical manifestation of EAE in C57BL/6 mice is a progressive ascending paralysis which starts in the tail and leads to forelimb paralysis. In mice with decreased IFN- signaling, EAE symptoms are atypical and characterized by defects in proprioception with axial rotatory movement and ataxia with little hind limb paralysis[1,2]. Typical EAE symptoms monitored daily using a standard clinical score ranging: 1) Loss of tail tone, 2) incomplete hind limb paralysis, 3) complete hind limb paralysis, 4) forelimb paralysis, 5) moribund/dead. Atypical EAE symptoms were scored as follows: 1) hunched appearance, slight head tilt, 2) severe head tilt, 3) slight axial rotation/staggered walking, 4) severe axial rotation/spinning, 5) moribund/dead. In our experiments, we observed that 60-80% of the IFN- R$^{-/-}$ mice exhibited atypical EAE (scoring described in the methods) and this was not affected by IFN- treatment.

Naïve Human CD4 T-cell Isolation. We obtained peripheral blood mononuclear cells from healthy donors (Stanford Blood Center) by centrifugation through Ficoll (Histopaque 1077; Sigma). CD4$^+$ T cells were isolated by magnetic bead depletion of CD19$^+$, CD14$^+$, CD56$^+$, CD16$^+$, CD36$^+$, CD123$^+$, CD8$^+$, T cell receptor- and T cell receptor-δ positive and glycophorin A–positive

cells on an AutoMACS instrument (Miltenyi Biotec). Naive CD45RA$^+$ T cells were obtained by depletion with anti-CD45RO and anti-CD25 magnetic beads (Miltenyi Biotec).

References

1. Wensky, A.K. et al. IFN-gamma determines distinct clinical outcomes in autoimmune encephalomyelitis. *J Immunol* **174**, 1416-23 (2005).
2. Stromnes, I.M., Cerretti, L.M., Liggitt, D., Harris, R.A. & Goverman, J.M. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat Med* **14**, 337-42 (2008).