Supplemental Table I – Genes upregulated in IL-6+IL-23 differentiated murine CD4 T cells compared to IL-6 differentiated CD4 T cells.

Gene*	Fold Change	p-value	Gene	Fold Change	p-value
Il22	22.07	$5.4X10^{-7}$	**Cd24a**	2.49	$1.2X10^{-4}$
Lgr4	12.24	$1.5X10^{-7}$	**Mt1**	2.48	$2.2X10^{-8}$
Enpp2	11.99	$1.1X10^{-5}$	**Procr**	2.47	$5.9X10^{-6}$
Gcnt2	8.39	$1.7X10^{-9}$	**Cxcr4**	2.44	$2.3X10^{-4}$
Plat	7.12	$2.4X10^{-8}$	**Ahr**	2.42	$1.5X10^{-3}$
Timp1	6.21	$5.1X10^{-10}$	**Alpk2**	2.42	$2.5X10^{-4}$
Tgm2	6.21	$3.5X10^{-8}$	**Cd24a**	2.49	$3.1X10^{-4}$
Lum	6.02	$1.2X10^{-6}$	**Cd163l1**	2.48	$2.2X10^{-3}$
Dab2	5.85	$4.6X10^{-10}$	**Gpr183**	2.38	$1.7X10^{-6}$
Tnfrsf8	5.65	$2.2X10^{-7}$	**Pigz**	2.37	$6.1X10^{-7}$
Smox	4.97	$9.9X10^{-6}$	**Spp1**	2.37	$8.4X10^{-4}$
Hlx	4.60	$3.6X10^{-7}$	**Il10**	2.28	$8.5X10^{-3}$
Ermn	4.45	$4.9X10^{-5}$	**Avpi1**	2.28	$1.4X10^{-5}$
Frmd4b	4.35	$2.5X10^{-9}$	**Ecm1**	2.27	$3.2X10^{-4}$
Mgll	4.12	$5.4X10^{-6}$	**Armcx6**	2.25	$3.4X10^{-6}$
C130090K23	4.06	$1.0X10^{-5}$	**Gnpda2**	2.25	$2.5X10^{-7}$
Serpinb1a	4.03	$8.2X10^{-5}$	**Mctp2**	2.23	$7.0X10^{-10}$
Casp6	4.01	$3.4X10^{-9}$	**Plac8**	2.20	$5.1X10^{-7}$
Zc3h12c	3.80	$3.3X10^{-7}$	**Paqr8**	2.20	$5.3X10^{-6}$
Ubd	3.79	$7.8X10^{-5}$	**Tiparp**	2.19	$7.2X10^{-4}$
Ltb4r1	3.67	$3.9X10^{-6}$	**Mdm1**	2.17	$1.0X10^{-5}$
Il1r1	3.13	$1.7X10^{-8}$	**Batf3**	2.15	$1.8X10^{-4}$
Gatm	3.02	$8.5X10^{-8}$	**Rorc**	2.15	$1.7X10^{-5}$
Serpinb5	2.98	$4.8X10^{-5}$	**Armcx3**	2.14	$8.9X10^{-6}$
Gp49a	2.94	$7.1X10^{-6}$	**Klre1**	2.13	$8.8X10^{-7}$
Cysltr1	2.90	$2.6X10^{-4}$	**Dgat1**	2.09	$1.1X10^{-3}$
Mt2	2.87	$2.1X10^{-9}$	**Prdm1**	2.09	$6.6X10^{-4}$
Ccr2	2.84	$1.1X10^{-6}$	**Tmem140**	2.09	$2.6X10^{-4}$
Gm14005	2.76	$1.9X10^{-6}$	**Armcx2**	2.08	$1.2X10^{-3}$
B4galnt4	2.58	$6.6X10^{-7}$	**C430003N24**	2.03	$1.1X10^{-4}$
Crispld2	2.56	$6.3X10^{-5}$	**Tiparp**	2.02	$2.0X10^{-4}$
Kctd12	2.56	$4.4X10^{-5}$	**Serpine1**	2.01	$2.0X10^{-4}$
Ccr5	2.51	$5.2X10^{-5}$			

*Selection criteria included a fold change >2 and p-value<0.01.

Supplemental Table II – Genes upregulated in IL-12+IL-23 differentiated murine CD4 T cells compared to IL-12 differentiated CD4 T cells.

Gene*	Fold-Change	p-value
Il17a	10.25	$2.8X10^{-5}$
Il22	8.45	$2.6X10^{-5}$
Enpp2	8.35	$5.2X10^{-5}$
Lum	3.93	$2.0X10^{-5}$
Cd163l1	2.15	$5.1X10^{-3}$
Ccr2	2.05	$5.0X10^{-5}$
Ermn	1.92	$7.5X10^{-4}$
Gatm	1.91	$2.3X10^{-5}$
Gcnt2	1.82	$1.0X10^{-3}$
Tnfrsf8	1.67	$9.5X10^{-3}$
Ltb4r1	1.57	0.017
Zc3h12c	1.45	0.017
Avpi1	1.41	0.012
Rps24	1.37	0.017
Dab2	1.34	0.013
Rora	1.34	0.019
Crispld2	1.33	$6.2X10^{-3}$
Casp6	1.29	0.014
Gstt1	1.29	$2.7X10^{-3}$
Gpld1	1.28	$1.9X10^{-3}$
Crhr1	1.27	$2.3X10^{-3}$
Sgms1	1.26	$7.9X10^{-3}$
Syt11	1.22	0.018
Calm2	1.21	$6.2X10^{-3}$
Chm	1.21	$7.3X10^{-3}$
Gnpda2	1.20	0.012

*Selection criteria was a p-value<0.02.

Supplemental Table III – Genes downregulated in IL-6+IL-23 differentiated murine CD4 T cells compared to IL-6 differentiated CD4 T cells.

Gene*	Fold-Change	p-value
Ccl20	0.34	$2.6X10^{-4}$
Lta	0.40	$1.6X10^{-4}$
LOC1000469	0.48	$1.7X10^{-4}$
Csf2	0.50	$7.3X10^{-3}$
Ifng	0.50	$7.1X10^{-5}$
Gprin3	0.53	$2.4X10^{-6}$
Nr4a3	0.53	$5.2X10^{-3}$
Tnfsf11	0.54	$5.8C10^{-3}$
Rgs16	0.56	$8.9X10^{-4}$
Penk	0.56	$1.6X10^{-3}$
Ccr6	0.57	$4.6X10^{-5}$
Lgals	0.57	$1.4X10^{-3}$
D630039A03	0.58	$2.7X10^{-3}$
Gata3	0.60	$1.3X10^{-3}$
Plagl1	0.60	$6.6X10^{-3}$
Egr3	0.60	$2.6X10^{-4}$
Dennd5a	0.61	$3.6X10^{-5}$
Il13	0.62	$4.7X10^{-3}$
Fam71b	0.63	$3.0X10^{-3}$
5830405N20	0.63	$6.3X10^{-4}$
Nrn1	0.64	$9.1X10^{-3}$
Fam26f	0.65	$1.0X10^{-3}$
Axl	0.65	$8.5X10^{-3}$
Gramd1b	0.66	$6.0X10^{-3}$

*Selection criteria included a fold change <0.66 and p-value<0.01.

Supplemental Table IV – Genes downregulated in IL-12+IL-23 differentiated murine CD4 T cells compared to IL-12 differentiated CD4 T cells.

Gene*	Fold-Change	p-value
Penk	0.66	0.012
Mfsd11	0.66	0.011
H3f3b	0.69	$2.3X10^{-3}$
Picalm	0.70	$7.0X10^{-3}$
Ahrr	0.71	0.010
2310014L17	0.72	$8.7X10^{-4}$
Hist1h3d	0.73	$1.6X10^{-3}$
Galnt10	0.76	$6.3X10^{-3}$
Kdm6b	0.77	$1.6X10^{-3}$
Dcakd	0.78	0.016
Nol11	0.78	$7.4X10^{-3}$
Ccnk	0.78	$1.5X10^{-3}$
Zc3h4	0.79	0.010
Mpp6	0.80	0.018
Cr1l	0.80	$8.5X10^{-3}$
Trim44	0.80	0.014
Snapc4	0.80	$2.8X10^{-3}$
Rbm4	0.80	0.013
Trmt61a	0.81	$3.2X10^{-3}$
Gm5303	0.82	0.011
Patz1	0.82	$5.2X10^{-3}$
Map3k4	0.82	$1.9X10^{-3}$
Mettl2	0.82	0.016
4930445K14	0.82	$3.4X10^{-3}$
Atox1	0.82	$2.7X10^{-3}$
Dus3l	0.83	$7.4X10^{-3}$

*Selection criteria was a p-value<0.02.

Supplemental Table V – Comparison of different STAT4 siRNA from different manufacturers in EAE.

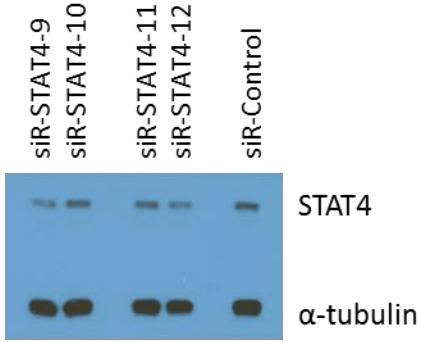
	---------- siR-STAT4 (Santa Cruz)* ----------			---------- siR-STAT4-9 (Dharmacon)** ----------		
	EAE Incidence (%)	EAE AUC***	Disease Course p-value****	EAE Incidence (%)	EAE AUC***	Disease Course p-value****
siRNA-NS	7/10 (70%)	27.25		10/16 (62.5%)	20.19	
siRNA-STAT4	4/10 (40%)	10.00	**0.0263**	5/13 (38.5%)	14.69	**0.0479**

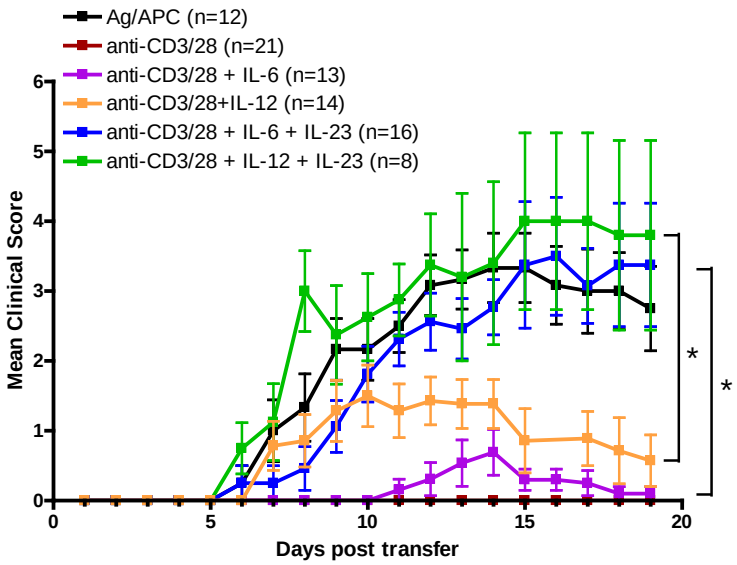
*siRNA pool used in Figure 5B from Santa Cruz Biotechnology compared to an independent siRNA produced by Dharmacon.

**siRNA-STAT4-9 manufactured by Dharmacon suppressed Stat4 protein levels by 40% when analyzed by Western blot (see image below).

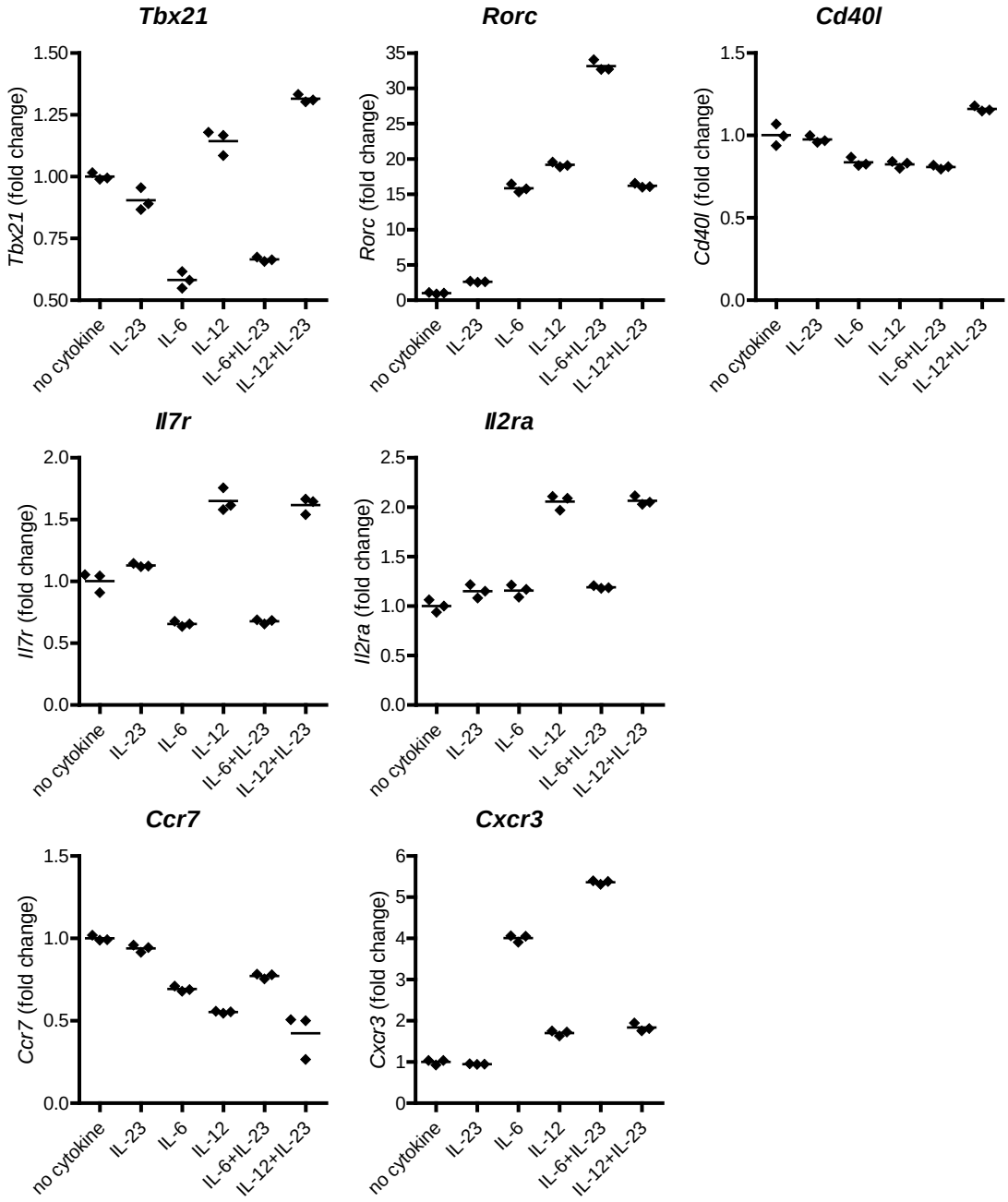
***Area under the curve for a 30 day analysis of EAE clinical scores.

****Mann-Whitney non-parametric analysis was used to analyze the difference in EAE clinical course.

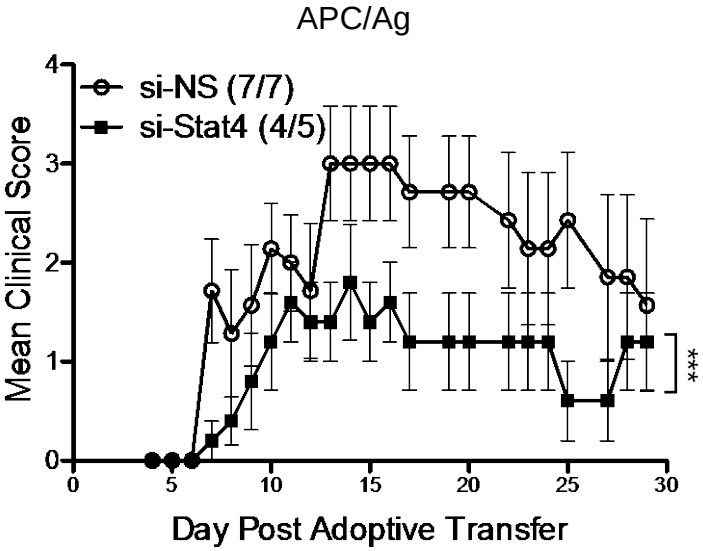




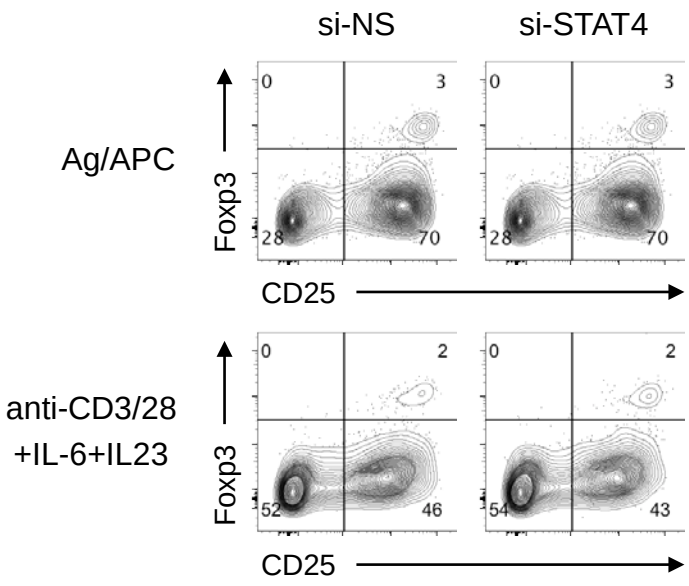
Supplemental Figure 1: IL-6+IL-23 and IL-12+IL-23 restore encephalitogenicity of myelin-specific CD4+ T cells differentiated with anti-CD3/CD28. This a composite of four experiments in which splenocytes from naïve Vα2.3/Vβ8.2 TCR Tg mice were activated *in vitro* with APC+MBPAc1-1 or anti-CD3/CD28 Ab in the presence of IL-6, IL-12 or the combination of IL-6+IL-12 or IL-12+IL-23 for 3 days prior to transfer into naïve B10.Pl mice ($5X10^6$ cell per mouse).



Supplemental Figure 2: Analysis of transcription factors, activation markers and chemokines associated with CD4 T cell activation. Naïve $CD4^+$ T cells were purified from WT B10.PL splenocytes and activated *in vitro* with anti-CD3/CD28 Ab and IL-23, IL-12, or IL-12+IL-23. Cells were collected at 60 h. RNA was extracted and real-time PCR was performed to determine gene expression. Fold change of gene expression was shown relative to no cytokine condition (mean±SEM).



Supplemental Figure 3: Suppressing STAT4 diminishes the encephalitogenicity of myelin-specific T cells. Splenocytes from naïve myelin-specific Vα2.3/Vβ8.2 TCR transgenic mice were transfected with either si-NS or si-Stat4 for 18 h. The cells were activated with MBP Ac1-11 peptide for 3 d and then adoptively transferred into naive B10.PL mice (10×106 cells per mouse). The number of mice with clinical signs / total number of mice in each group is shown as follows: si-NS (7/7); and si-STAT4 (4/5). There was a statistically significant reduction in EAE as determined by Mann-Whitney. The data is representative of 3 independent experiments.



Supplemental Figure 4: Analysis of Foxp3 and CD25 in si-NS and si-STAT4 transfected CD4 T cells. Splenocytes from naïve Vα2.3/Vβ8.2 TCR Tg mice were transfected with either si-NS or si-Stat4 for 18 h. The cells were activated with MBP Ac1-11 peptide for 3 d (top panel) or activated with anti-CD3/CD28 Ab plus IL-6+IL-23 for 60 h (low panel). The CD4 T cells were analyzed by flow cytometry for CD25 and Foxp3.