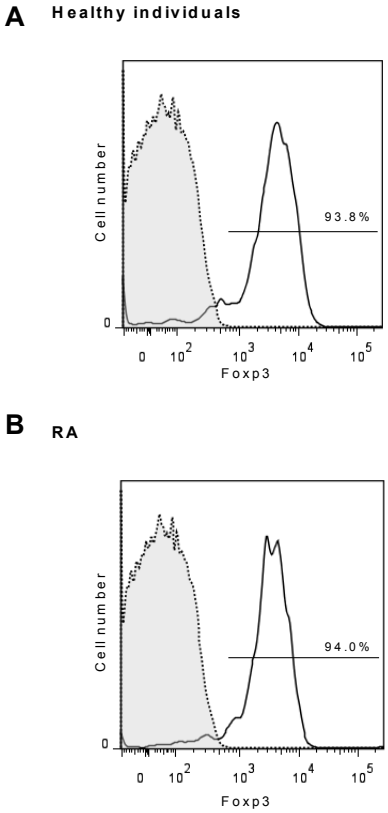
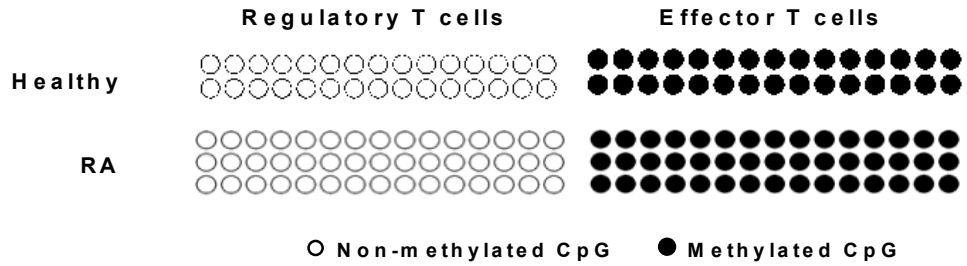
SUPPLEMENTAL FIGURES



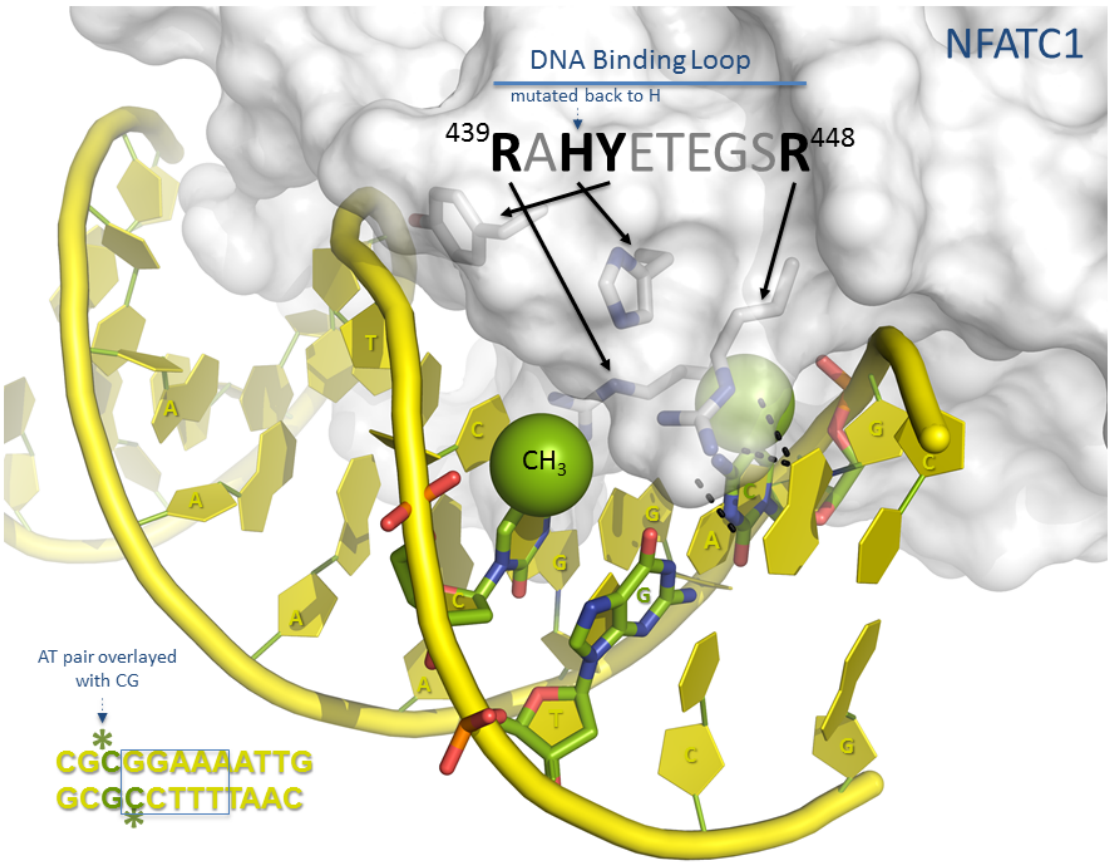
Supplementary Figure 1: Cell sorting purity of healthy and RA patients

$CD4^+$ cells were isolated using MACS CD4 T cell negative cell isolation kit. Tregs and Teffs were isolated by FACS sorting using CD25 and CD127 antibodies. Expression of Foxp3 is shown in a representative donor from **A.** healthy and **B.** RA for $CD4^+CD25^+CD127^-$ (Treg sorted) populations.



Supplementary Figure 2: Methylation analysis of the TSDR indicates that CD25^{+}CD127^{-} cells isolated from healthy individuals and RA donors are highly pure natural Tregs

Methylation analysis of the TSDR from male healthy individuals and RA patients. CD25^{+}CD127^{-} and CD25^{-}CD127^{+} cells were FACS sorted prior to genomic DNA extraction. Genomic DNA was then bisulphite converted, PCR amplified and then sequenced. Sequences were analysed by QUMA which determined the methylation status of each CpG. Each circle represents one CpG within the TSDR region and each row represents one donor. Open circles represent demethylated CpG and filled circles represent methylated CpG.



Supplementary Figure 3: Modelling the effect of methylation on the ability of NFAT2 to bind to DNA

The NMR structure of NFAT2 bound to DNA was obtained from Zhou *et al* [23], Solution structure 1A66.pdb, state 12. The DNA binding loop was *in silico* mutated from Arg to His to accurately reflect the native conformation of NFAT2. The DNA oligonucleotide sequence was altered by overlaying a C and G base on to A and T bases to accurately reflect the sequence of methylation in the CTLA-4 promoter. Black arrows denote the functional amino acids interacting with the DNA. Original DNA bases (Yellow lettering), modified bases (Green lettering). Dashed lines **–** 3 hydrogen bonds between R^{448} and G in current configuration. The figure was generated using PyMOL software (Schrödinger, USA).

Supplementary Table 1. Patient demographics of RA patients

Sex	Age (y)	Duration of symptoms	Anti-CCP	RF	DAS28	Smoker
Female	56	1 month	-	-	6.64	No
Female	53	2 years	+	-	5.40	No
Female	74	4 months	+	+	5.42	Prev
Male	54	4 months	+	+	5.80	No
Female	74	8 months	+	+	5.58	No
Female	48	6 months	+	+	5.68	Prev
Female	45	8 months	+	+	6.69	No
Female	43	2 years	+	+	4.27	No
Female	44	4 months	-	+	5.79	No
Male	38	1 year	-	-	5.10	Prev
Female	60	6-7 weeks	+	+	5.16	No
Female	48	2 years	+	-	5.94	Prev
Male	43	2 years	+	+	4.27	No
Female	72	10 months	+	+	4.45	No
Female	28	6 months	+	+	5.00	No
Female	44	2 months	+	+	4.60	No
Female	50	6 months	+	+	5.13	Prev
Female	60	6 months	n.a.	+	7.50	No

Demographics for all rheumatoid arthritis (RA) clinical peripheral blood samples used in this study. All patients were diagnosed on day of sample taken and were drug naïve. Where available, rheumatoid factor status (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP) staus, DAS28 score, and smoking history were obtained. n.a. = not available

Supplementary Table 2: Bisulphite specific primers

Primer (bp from ATG site)	Forward primer	Reverse primer
1659 - 1221	TTTTGAGGGTAGGAATATTTGT	TTTAACCTATACCCCAACATTC
1235 - 744	GGGTATAGGTTAAAGAGGGAGG	AAAAAATCCAAAAAAAAACCAA
767 - 362	GTTGGTTTTTTTTTGGATTTTT	TACAAAAATCCTCCTAAATCCC
210 - 77	AAATTGGGCTTTAGGAGGATTT	TAAACCCACACAAAATCAAAAA

The forward and reverse primers used to determine the methylation state of the CpGs within the *Ctla4* promoter.