

Supplemental Material

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Sequence motifs associated with DMR showing enhancer activity**Supplemental Figures**

Supplemental Figure S1:

Comparison of methylation states in T cells and mRNA expression data of associated genes.

(A) The box plots show the distribution of mRNA expression ratios (freshly sorted T cells, left panel; *in vitro* expanded T cells, right panel) conditional on the methylation status of the associated DMR. The red lines denote medians, boxes the interquartile ranges, and whiskers the 5th and 95th percentiles. Pair wise comparisons of mRNA expression ratios associated with DMR hypermethylated in Tconv or Treg cells are significant (** $P < 0.001$, * $P < 0.01$, Mann–Whitney U test, two-sided). (B) Box plots are shown as in (A) with DMR divided into the two major position classes: intergenic and intragenic. Differences between inter- and intragenic pairs of the same cell type and the same DMR status were not significant ($P > 0.05$, Mann–Whitney U test, two-sided).

Supplemental Figure S2:

Comparison of methylation states in T cells and CD34+ progenitor cells. The methylation states of individual CpG residues obtained by MALDI-TOF MS were compared between CD34+ progenitor cells and the highest (left diagram) or the lowest T cell methylation value (right diagram). Differentially methylated CpG residues that acquire methylation (compared to the progenitor cell) should be found in the upper left corner of the left diagram, whereas CpG residues that are demethylated (compared to the progenitor cell) should be found in the lower right corner of the right diagram.

Supplemental Figure S3:

Chromatin modification and CTCF binding patterns across the *FOXP3* gene locus. Tracks and heat maps are shown as described in the legend of Figure 2.

Supplemental Figure S4:

Chromatin modification and CTCF binding patterns across the *CD40LG* gene locus. Tracks and heat maps are shown as described in the legend of Figure 2.

Supplemental Figure S5:

Chromatin modification and CTCF binding patterns across the *IFNG* gene locus. Tracks and heat maps are shown as described in the legend of Figure 2.

Supplemental Figure S6:

Chromatin modification and CTCF binding patterns across the *LRRC32* gene locus. Tracks and heat maps are shown as described in the legend of Figure 2.

Additional Supplemental Material:

UCSC Genome Browser track files:

CpG_Index.gz

CTCF_Tconv.gz

CTCF_Treg.gz

H3K4mono_Tconv.gz

H3K4mono_Treg.gz

H3K4di_Tconv.gz

H3K4di_Treg.gz

H3K4tri_Tconv.gz

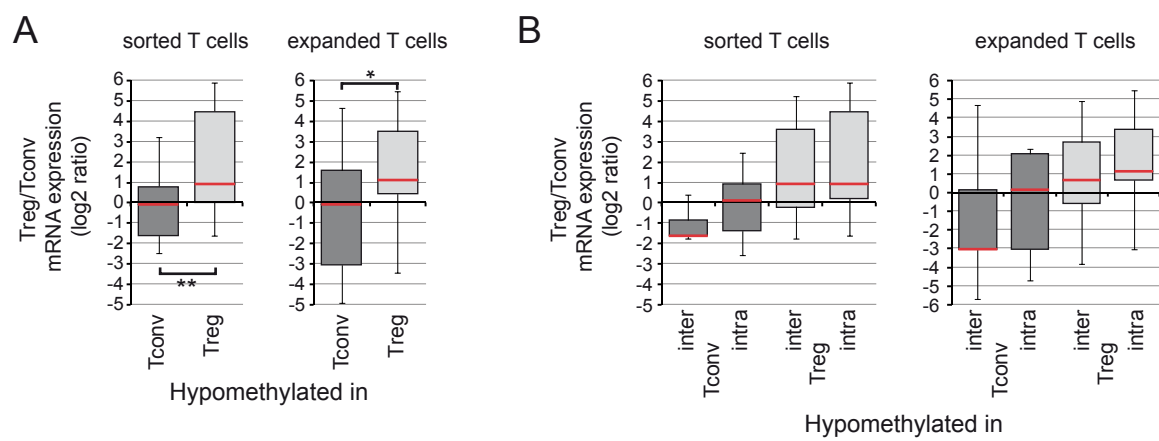
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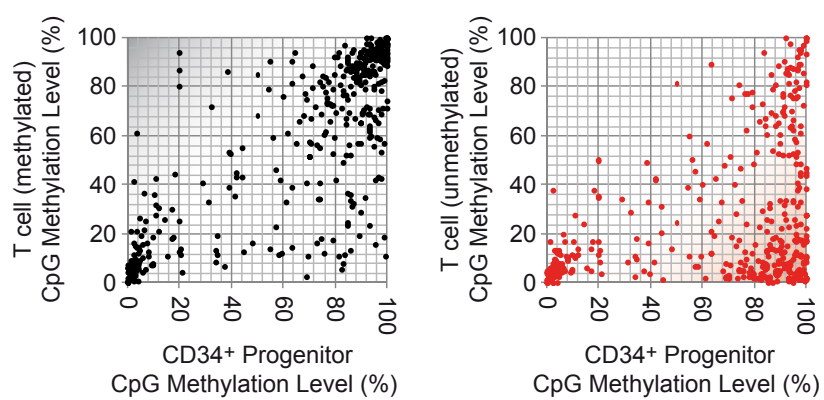
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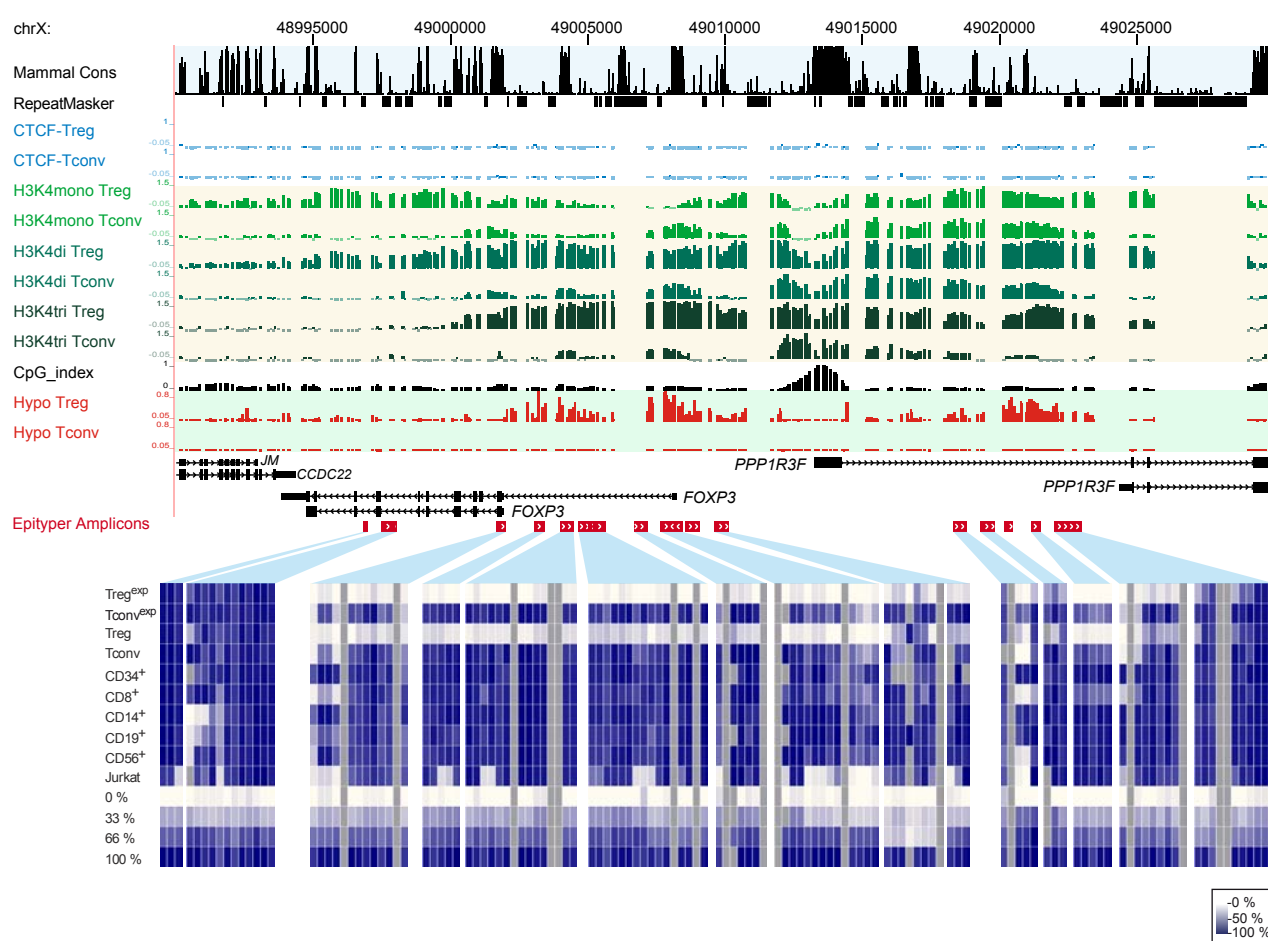
Supplemental Figure S1



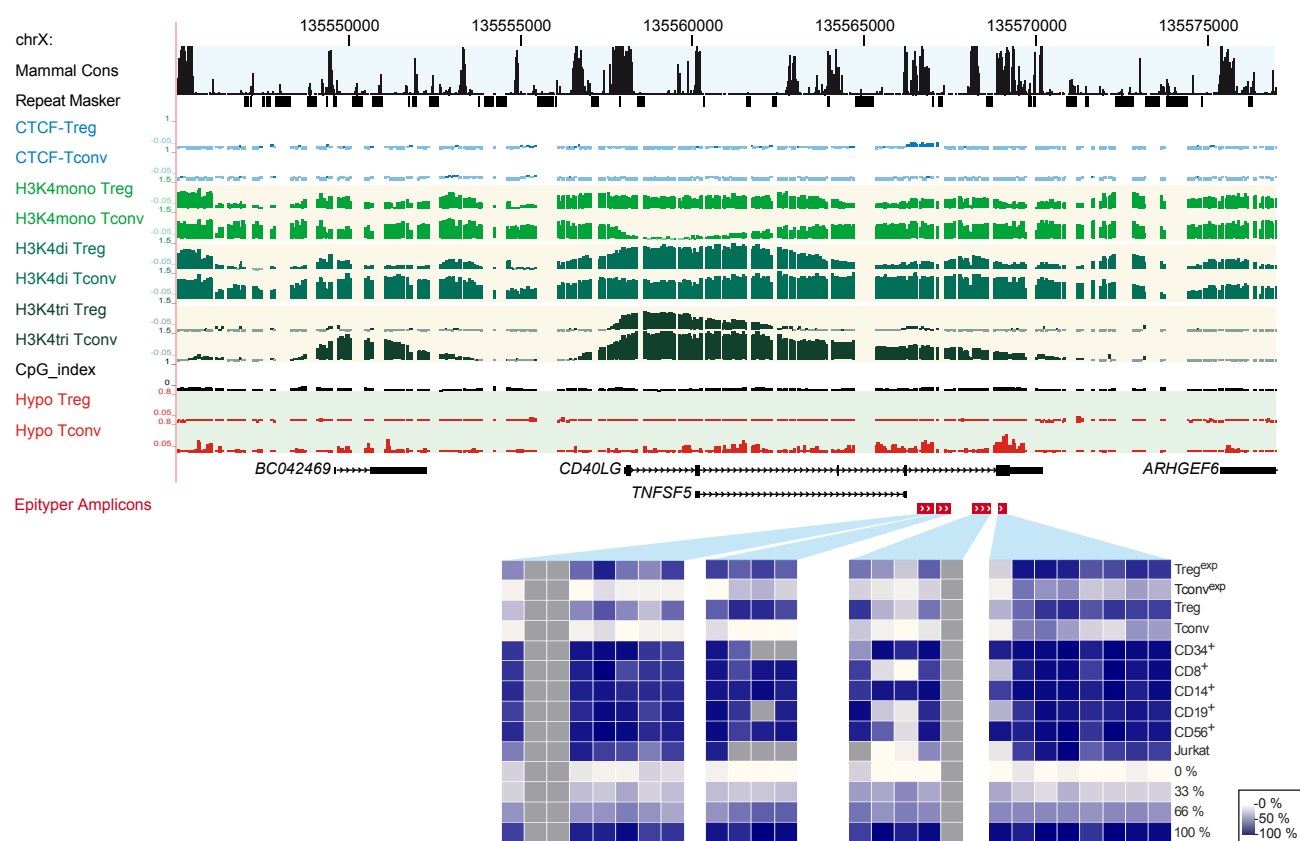
Supplemental Figure S2



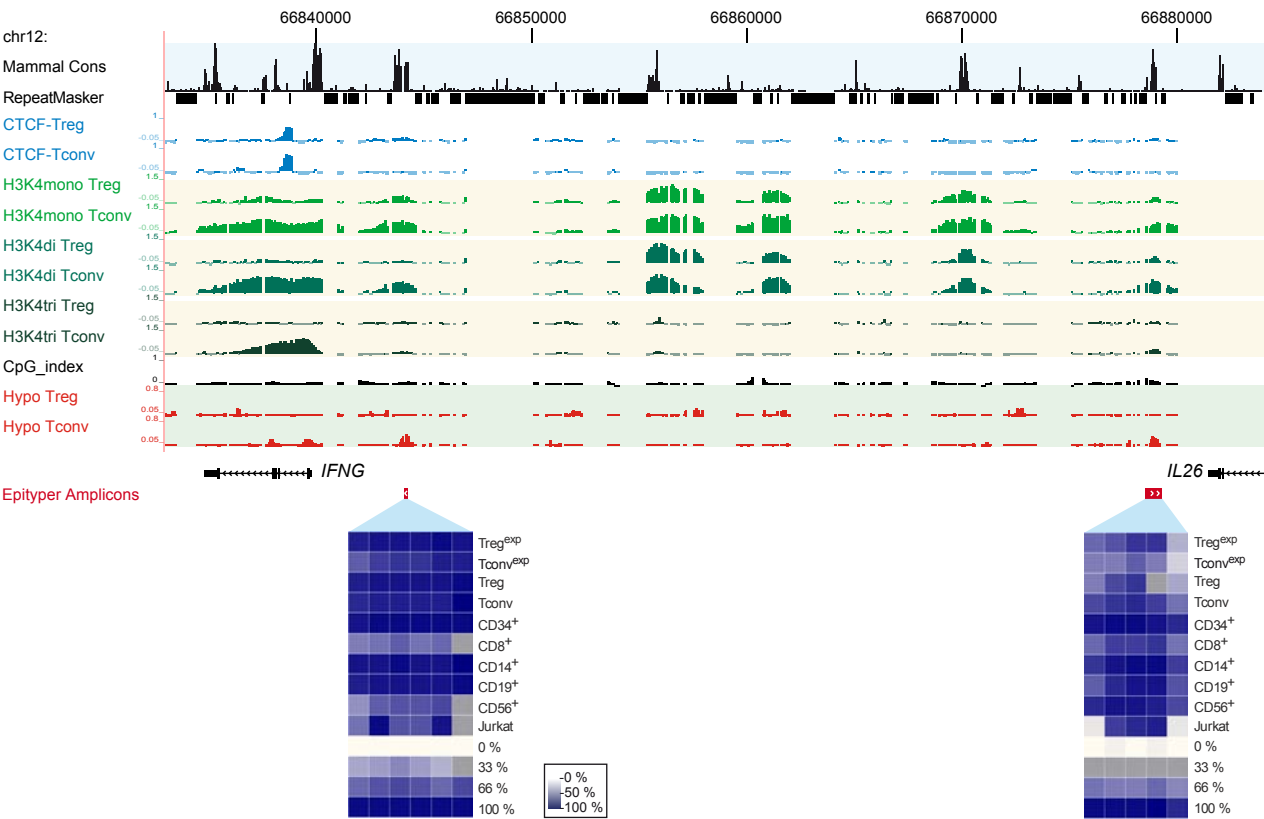
Supplemental Figure S3



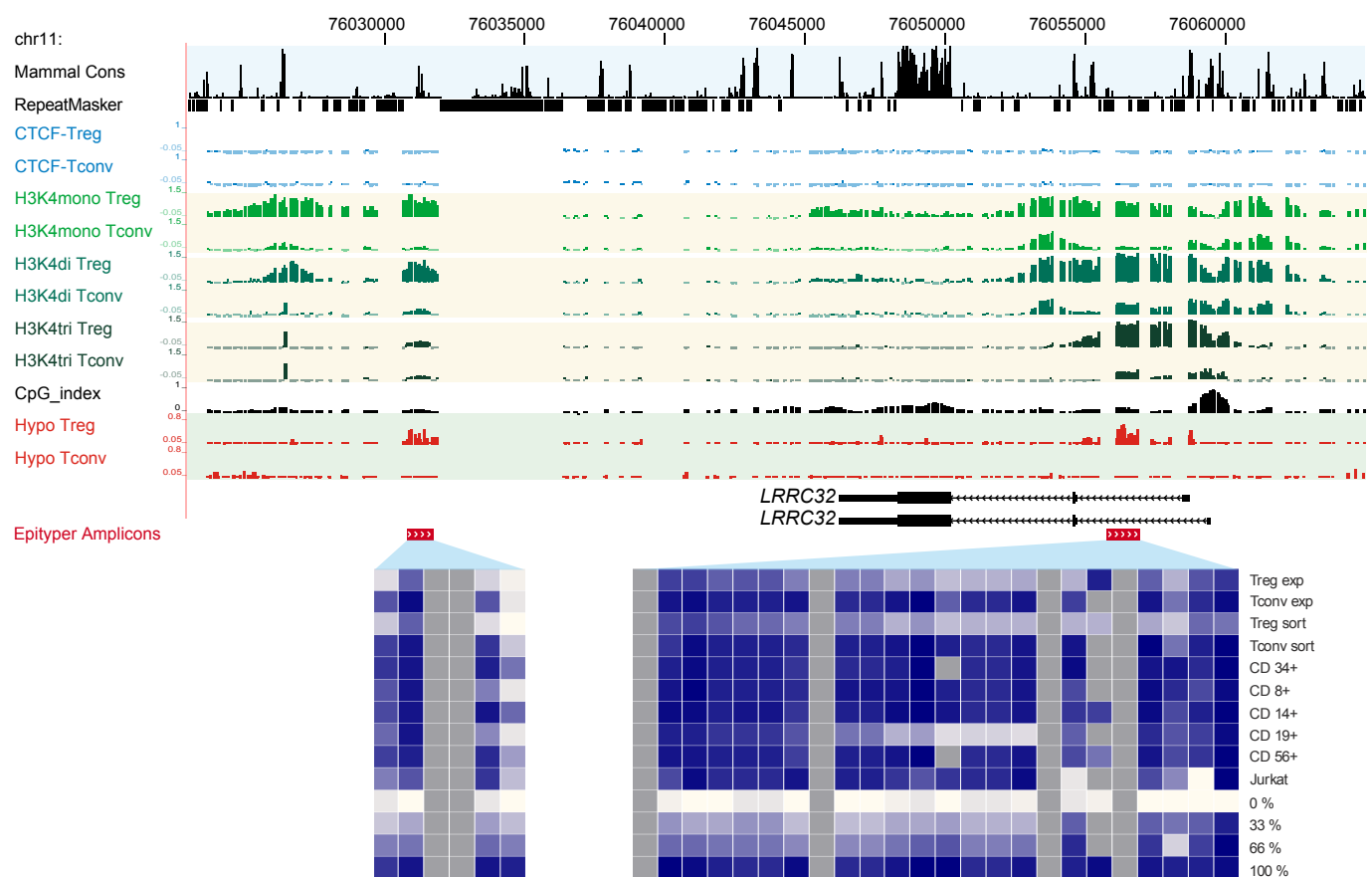
Supplemental Figure S4



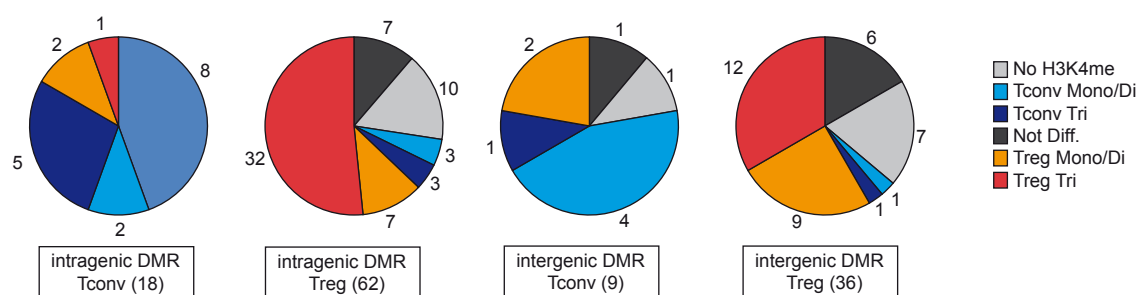
Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7



Supplemental Table S1
Genomic regions selected for Treg/Tconv cell DMR screening

Gene Symbol	Location (Mar. 06 (hg18) Assembly)	Region Size
ADC	chr1:33,280,000-33,380,000	100000
ANK3	chr10:61,350,000-62,200,000	850000
ANXA1	chr9:74,900,000-75,030,000	130000
CD40LG	chrX:135,520,000-135,580,000	60000
CHD7	chr8:61,700,000-62,000,000	300000
CYSLTR1	chrX:77,300,000-77,600,000	300000
GZMA	chr5:54,320,000-54,450,000	130000
HOP	chr4:57,170,000-57,300,000	130000
ID2	chr2:8,680,000-8,770,000	90000
IFNG	chr12:66,780,000-66,880,000	100000
IL18RAP	chr2:102,380,000-102,450,000	70000
MGC33556	chr1:44,900,000-44,980,000	80000
NELL2	chr12:43,130,000-43,680,000	550000
TARP/CD3G	chr7:38,230,000-38,340,000	110000
IL4	chr5:132,000,000-132,060,000	60000
ADAMTS4	chr1:159,400,000-159,460,000	60000
ARG1	chr6:131,860,000-132,000,000	140000
DYX1C1	chr15:53,430,000-53,610,000	180000
FCRL3	chr1:155,840,000-155,960,000	120000
FOXP3	chrX:48,970,000-49,030,000	60000
IL1R2/IL1R1	chr2:101,870,000-102,175,000	305000
LRRC32	chr11:76,000,000-76,150,000	150000
RTKN2	chr10:63,580,000-63,780,000	200000
F5/SELP	chr1:167,730,000-167,900,000	170000
CAMTA1/TNFRSF9/UTS2	chr1:7,600,000-7,940,000	340000
WDFY4	chr10:49,620,000-49,900,000	280000
WNT10A	chr2:219,443,000-219,490,000	47000
IKZF2	chr2:213,500,000-213,850,000	350000
NOG	chr17:51,970,000-52,070,000	100000
IL7R	chr5:35,850,000-35,940,000	90000
PTPRC	chr1:196,800,000-197,020,000	220000
TP53INP1	chr8:96,000,000-96,045,000	45000
IL6R	chr1:152,590,000-152,720,000	130000
AMOTL2	chr3:135,500,000-135,630,000	130000
PKD2	chr4:89,120,000-89,230,000	110000
LGALS3	chr14:54,605,000-54,685,000	80000
LCAM3	chr9:132,800,000-132,980,000	180000
PERP	chr6:138,420,000-138,520,000	100000
S100Z	chr5:76,165,000-76,270,000	105000
CTLA4	chr2:204,390,000-204,490,000	100000
IL2RA	chr10:6,060,000-6,170,000	110000
IL2RB	chr22:35,835,000-35,905,000	70000
ENTPD1	chr10:97,440,000-97,670,000	230000
NT5E	chr6:86,170,000-86,270,000	100000
PDE4D	chr5:58,250,000-59,850,000	1600000
ZEB1	chr10:31,600,000-31,900,000	300000
FCER1G	chr1:159,445,000-159,460,000	15000
TRAT1	chr3:109,990,000-110,090,000	100000
FAIM3	chr1:205,128,000-205,168,000	40000
MAP3K5	chr6:136,915,000-137,180,000	265000
GJB6	chr13:19,665,000-19,765,000	100000

Supplemental Table S1 (continued)
Genomic regions selected for Treg/Tconv cell DMR screening

Gene Symbol	Location (Mar. 06 (hg18) Assembly)	Region Size
ACTB*	chr7:5,520,000-5,570,000	50000
B2M	chr15:42,760,000-42,810,000	50000
LTB	chr6:31,636,000-31,686,000	50000
SEPT9	chr17:72,740,000-73,040,000	300000
HPRT1	chrX:133,390,000-133,490,000	100000
UBC	chr12:123,950,000-123,980,000	30000
KLF2	chr19:16,265,000-16,325,000	60000
CD2	chr1:117,070,000-117,130,000	60000
SPI1	chr11:47,330,000-47,380,000	50000
TLR4	chr9:119,460,000-119,550,000	90000
TFEC	chr7:115,350,000-115,500,000	150000
CD14	chr5:139,980,000-140,000,000	20000
CD19	chr16:28,840,000-28,870,000	30000
NCAM1	chr11:112,300,000-112,660,000	360000
GATA1	chrX:48,515,000-48,547,000	32000
GATA4	chr8:11,500,000-11,660,000	160000
CSF1R	chr5:149,400,000-149,490,000	90000
HOXA9	chr7:27,080,000-27,220,000	140000

* Control loci are boxed in green.

Supplemental Table S2
Location and properties of DMR detected by MCIP

Gene Symbol and Gene ID	Log ₂ ratio freshly sorted Treg/Tconv ^a	Log ₂ ratio expanded Treg/Tconv	Probe ID	Chr. Location	Distance from TSS	Relative Position to Gene Transcripts ^b	Hypomethylated T cell type	Epityper validation of MCIP results	CpG content class ^c	Overlap with PhastCons ^d	Overlap with DHS in CD4+ T cells ^e	H3K4 Methylation ^f Status
VAMP3 9341	0.01	0.33	A_24_P370887	chr1:7694083-7694556	-59600	intra	Treg		LCR			Treg Mono/Di
				chr1:7696779-7696912	-57074	intra	Tconv		LCR			no H3K4me
				chr1:7742983-7743147	-10855	intra	Tconv		LCR			no H3K4me
PER3 8863	0.12	-0.06	A_24_P291231	chr1:7763681-7763839	-3209	intra	Tconv		LCR			Tconv Mono/Di
				chr1:7780936-7781346 ^a	14172	intra	Tconv		LCR	yes		no H3K4me
UTS2 10911	4.66	5.61	A_23_P63343	chr1:7836042-7836108	-393	5-proximal	Tconv		LCR			no H3K4me
TNFRSF9 3604	3.58	4.06	A_23_P51936	chr1:7921606-7922186	1574	intra	Treg		LCR			Treg Tri
				chr1:7923689-7923832	-291	5-proximal	Treg	yes	LCR			Treg Tri
				chr1:7931900-7932340	-8650	inter	Treg		ICR	yes	DHS	Treg Mono/Di
KIF2C 11004	0.52	-3.02	A_23_P34788	chr1:44978856-44978930	772	intra	Treg		LCR		DHS	Tconv Tri
PTPRC 5788	-0.27	0.46	A_23_P125451	chr1:196900673-196900751	25919	intra	Treg		LCR		DHS	Treg Tri
				chr1:196941013-196941144	66286	intra	Treg		LCR		DHS	Not diff.
FAIM3 9214	-0.44	3.39	A_23_P138125	chr1:205146071-205146415	15666	intra	Treg		LCR		DHS	Not diff.
				chr1:205159845-205160036	1968	intra	Treg		LCR		DHS	Treg Tri
				chr1:205160872-205161211	867	intra	Treg		LCR		DHS	Treg Tri
				chr1:205164864-205165172	-3109	inter	Treg		LCR		DHS	Treg Mono/Di
ID2 3398	-2.41	-6.67	A_32_P69368	chr2:8726198-8726662	-13206	inter	Treg		LCR			no H3K4me
				chr2:8735102-8735444	-4363	inter	Treg	yes	LCR	yes	DHS	Tconv Mono/Di
				chr2:8740559-8740969	1128	intra	Treg	no	ICR	yes	DHS	Tconv Tri
IL1R2 7850	3.69	4.67	A_23_P79398	chr2:101984548-101985058	2913	intra	Treg		LCR			Treg Mono/Di
				chr2:102045612-102046101	63967	inter	Treg		LCR			Not diff.
CTLA4 1493	2.36	1.96	A_23_P102481	chr2:204408646-204409889	-31484	inter	Treg	yes	LCR	yes		Treg Tri
				chr2:204426657-204428059	-13394	inter	Treg	yes	LCR	yes	DHS	Treg Tri
				chr2:204443336-204444178	3005	intra	Treg	yes	LCR	yes	DHS	Treg Tri
				chr2:204446111-204447000	5804	intra	Treg	yes	LCR	yes	DHS	Treg Tri
				chr2:204460103-204460326	19463	inter	Treg		LCR			Treg Mono/Di
IKZF2 22807	4.49	3.84	A_32_P114284	chr2:213658721-213658870	64554	intra	Treg		LCR	yes		Treg Mono/Di
				chr2:213680605-213681119	42488	intra	Treg		ICR	yes		Treg Tri
				chr2:213688110-213688358	35116	intra	Treg		LCR			Treg Mono/Di
				chr2:213697294-213697468	25969	intra	Treg	yes	LCR			Treg Tri
				chr2:213706776-213707454	16235	intra	Treg		LCR			Treg Tri
				chr2:213722578-213724345	-112	5-proximal	Treg	no	LCR	yes	DHS	Treg Tri
				chr2:213725364-213725750	-2207	inter	Treg		ICR	yes		Treg Tri
				chr2:213726946-213727686	-3966	inter	Treg	yes	LCR		DHS	Treg Tri
				chr2:213730460-213730581	-7171	inter	Treg		LCR			Treg Mono/Di
				chr2:213733406-213733693	-10200	inter	Treg		LCR			no H3K4me
SPAG16 79582	0.04	-0.21	A_23_P67785	chr2:213803183-213803316	-54169	inter	Treg		ICR			Treg Mono/Di
				chr2:213809847-213810325	-47333	inter	Treg	yes	LCR	yes		Treg Tri

Supplemental Table S2 (continued)
Location and properties of DMR detected by MCIP

Gene Symbol and Gene ID	Log ₂ ratio freshly sorted Treg/Tconv	Log ₂ ratio expanded Treg/Tconv	Probe ID	Chr. Location	Distance from TSS	Relative Position to Gene Transcripts ^b	Hypomethylated T cell type	Epityper validation of MCIP results	CpG content class ^c	Overlap with PhastCons ^d	Overlap with DHS in CD4+ T cells ^e	H3K4 Methylation ^f Status
<i>HOPX</i> 84525	-0.83	-3.75	A_23_P254507	chr2:213813204-213813362	-44136	inter	Treg		LCR	no	no	Treg Tri
				chr2:213814671-213814814	-42676	inter	Treg		LCR	no	no	Treg Tri
				chr4:57215993-57216190	26128	intra	Tconv		ICR	no	DHS	Tconv Tri
<i>PKD2</i> 5311	0.11	1.79	A_24_P106112	chr4:57242355-57242622	-269	5-proximal	Tconv		LCR	yes	DHS	ND
				chr4:89148733-89149406	1160	intra	Treg		LCR	yes	DHS	Treg Tri
				chr5:58263564-58263732	348985	inter	Treg		LCR	no	no	no H3K4me
<i>RAB3C</i> 115827	0.91	1.49	A_23_P44794	chr5:58265981-58266738	351697	inter	Treg		LCR	yes	DHS	ND
				chr5:58369473-58369731	454939	intra	Treg		LCR	yes	DHS	Tconv Tri
				chr5:58371799-58371934	457204	intra	Tconv		LCR	no	DHS	Tconv Tri
<i>F2RL1</i> 2150	0.61	1.72	A_23_P58835	chr5:58374023-58374163	459430	intra	Treg		LCR	yes	DHS	Tconv Mono/Di
				chr5:76165074-76165598	14707	intra	Tconv		LCR	yes	DHS	no H3K4me
				chr6:136915800-136915872	-2583	inter	Treg		LCR	no	no	no H3K4me
<i>MAP7</i> 9053	-0.40	-0.81	A_24_P98021	chr6:137124814-137124977	30453	intra	Treg		LCR	no	DHS	Treg Tri
				chr6:137139643-137139811	15622	intra	Tconv		LCR	no	no	Treg Mono/Di
				chr6:137156228-137156491	-1011	inter	Treg		LCR	no	DHS	ND
<i>MAP3K5</i> 4217	1.31	2.40	A_23_P134125	chr6:138458928-138459115	11227	intra	Tconv		LCR	no	no	no H3K4me
				chr6:138468457-138468554	1743	intra	Tconv		LCR	no	no	Treg Tri
				chr7:27103645-27103925	-1645	intra	Treg		LCR	yes	no	ND
<i>PERP</i> 64065	1.96	2.35	A_23_P214950	chr7:27146342-27146410	-26	5-proximal	Tconv		ICR	yes	no	no H3K4me
				chr7:27184543-27184956	1449	intra	Tconv		LCR	yes	no	no H3K4me
				chr7:27208821-27209379	-2850	intra	Treg		LCR	no	no	no H3K4me
<i>HOXA1</i> 3198	0.91	1.88	A_23_P31306	chr7:27212390-27212560	-6225	inter	Treg		ICR	yes	no	no H3K4me
				chr8:11580580-11581129	-18270	intra	Treg		LCR	yes	no	no H3K4me
				chr8:11583722-11583857	-15335	intra	Treg	no	LCR	no	no	no H3K4me
<i>HOXA3</i> 3200	0.91	1.49	A_23_P501538	chr8:11601942-11602190	2941	intra	Treg		LCR	no	no	no H3K4me
				chr8:11607397-11607483	8315	intra	Treg		LCR	no	no	no H3K4me
				chr8:11639562-11639728	40520	intra	Treg		LCR	no	no	no H3K4me
<i>HOXA10</i> 3206	0.91	1.88	A_23_P31306	chr8:61727910-61728135	-25892	inter	Treg		LCR	no	DHS	ND
				chr8:61756004-61756256	2215	intra	Tconv		ICR	no	DHS	Tconv Tri
				chr8:61759841-61760294	6153	intra	Treg		LCR	no	DHS	Tconv Mono/Di
<i>HOXA13</i> 3209	0.91	1.88	A_23_P31306	chr8:61761414-61761574	7579	intra	Treg		LCR	no	no	ND
				chr8:61776071-61776160	22201	intra	Tconv		LCR	no	DHS	Tconv Mono/Di
				chr8:61942900-61943092	189081	inter	Tconv		LCR	no	DHS	Tconv Mono/Di
<i>GATA4</i> 2626	0.79	1.13	A_24_P932785	chr8:61973666-61973792	219814	inter	Tconv		LCR	no	no	no H3K4me
				chr8:61979393-61979852	225708	inter	Tconv		LCR	no	no	Tconv Mono/Di
				chr8:61982195-61982630	228498	inter	Treg		ICR	no	no	ND
<i>CHD7</i> 55636	-1.62	-3.04	A_24_P58381	chr8:61984364-61984705	230620	inter	Treg		LCR	yes	DHS	Tconv Tri
				chr8:61985582-61985644	231698	inter	Tconv		ICR	yes	DHS	Tconv Tri

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<i>TP53INP1</i> 94241	0.93	1.01	A_23_P168882	chr8:61987949-61988359	234239	inter	Tconv		LCR	yes	DHS	Tconv Mono/Di
				chr8:96009841-96010902	-2996	intra	Treg	yes	LCR	yes	DHS	Treg Tri
				chr8:96028605-96029654	1630	intra	Treg		LCR	yes	DHS	Treg Tri
				chr8:96042041-96042225	-11373	intra	Treg		LCR	yes	DHS	ND
<i>IL15RA</i> 3601	0.70	0.54	A_23_P138680	chr10:6086021-6086185	-26153	inter	Tconv		LCR	no		Treg Mono/Di
<i>IL2RA</i> 3559	5.87	0.67	A_23_P127288	chr10:6119192-6119913	24725	intra	Treg	yes	LCR	no	DHS	Treg Tri
				chr10:6121979-6122460	22058	intra	Treg	yes	LCR	no		Treg Tri
				chr10:6128278-6128419	15929	intra	Treg		LCR	no	DHS	Treg Tri
				chr10:6129214-6129562	14890	intra	Treg		LCR	no	DHS	Treg Tri
				chr10:6134052-6134250	10127	intra	Treg		LCR	no	DHS	Treg Tri
				chr10:6136503-6137025	7514	intra	Treg		LCR	no		Treg Tri
				chr10:6137878-6138428	6125	intra	Treg		LCR	no		Treg Tri
				chr10:6139024-6140346	4593	intra	Treg		LCR	yes		Treg Tri
				chr10:6141939-6142194	2211	intra	Treg		LCR	no		Treg Tri
				chr10:6153181-6153487	-9056	inter	Treg	yes	LCR	no		Treg Tri
<i>RBM17</i> 84991	-0.14	-0.54	A_23_P423315	chr10:6154347-6154892	-10342	inter	Treg	yes	LCR	no	DHS	Treg Tri
				chr10:6164740-6165011	-6148	inter	Treg		LCR	no		Treg Tri
				chr10:6168811-6168881	-2178	inter	Treg		LCR	no	DHS	Treg Tri
<i>ZEB1</i> 6935	-0.01	-0.60	A_23_P202013	chr10:31650245-31650763	2357	intra	Treg		ICR	yes	DHS	ND
<i>LRRC18</i> 474354	0.13	-0.29	A_23_P35494	chr10:49833171-49833344	-40975	intra	Tconv		LCR	yes		no H3K4me
<i>ANK3</i> 288	-3.25	-3.52	A_23_P301530	chr10:61910645-61910927	-91151	intra	Tconv		LCR	no		no H3K4me
<i>RTKN2</i> 219790	4.39	5.47	A_24_P13041	chr10:63631396-63631747	66762	intra	Treg		LCR	yes		Treg Mono/Di
				chr10:63669692-63669980	28498	intra	Treg		LCR	no	DHS	Treg Tri
<i>ENTPD1</i> 953				chr10:97638620-97638752	132774	intra	Treg		LCR	no		no H3K4me
<i>FANK1</i> 92565	6.32	5.84	A_23_P115785	chr10:127661157-127661287	86070	intra	Treg		LCR	no		Treg Mono/Di
				chr10:127674302-127675105	99552	intra	Treg		LCR	no		Treg Tri
<i>LRRC32</i> 2615	4.96	5.47	A_24_P389916	chr11:76018362-76018714	40900	inter	Treg		LCR	yes		Treg Mono/Di
				chr11:76030870-76031550	28228	inter	Treg	yes	LCR	yes		Treg Tri
				chr11:76056081-76056862	2966	intra	Treg	yes	LCR	no		Treg Tri
				chr11:76058705-76058782	694	intra	Treg		LCR	yes		Treg Tri
<i>NCAM1</i> 4684	0.91	1.26	A_23_P1740	chr11:112345302-112345374	7987	intra	Treg		LCR	no		Tconv Mono/Di
				chr11:112350888-112350995	13591	intra	Treg		LCR	yes		no H3K4me
				chr11:112443435-112443585	106159	intra	Treg		LCR	yes		no H3K4me
<i>TTC12</i> 54970	-0.45	1.08	A_23_P24535	chr11:112638456-112638589	-51992	intra	Treg		LCR	yes		Treg Mono/Di
<i>NELL2</i> 4753	-4.64	-6.42	A_23_P10025	chr12:43288526-43288688	267807	intra	Treg		LCR	no		no H3K4me
<i>IFNG</i> 3458	-1.90	-6.25	A_23_P151294	chr12:66844066-66844304	-4395	inter	Tconv	yes	LCR	yes		Tconv Mono/Di
<i>IL26</i> 55801	-0.06	-5.23	A_23_P128503	chr12:66878821-66878935	26959	inter	Tconv	yes	ICR	yes	DHS	ND

Supplemental Table S2 (continued)
Location and properties of DMR detected by MCIP

Gene Symbol and Gene ID	Log ₂ ratio freshly sorted Treg/Tconv	Log ₂ ratio expanded Treg/Tconv	Probe ID	Chr. Location	Distance from TSS	Relative Position to Gene Transcripts ^b	Hypomethylated T cell type	Epityper validation of MCIP results	CpG content class ^c	Overlap with PhastCons ^d	Overlap with DHS in CD4 ⁺ T cells ^e	H3K4 Methylation ^f Status
<i>GJB2</i> 2706	0.91	-3.44	A_23_P204947	chr13:19666918-19667157	-1961	inter	Treg		LCR	no	DHS	Treg Mono/Di
<i>GJB6</i> 10804	1.79	2.18	A_23_P2745	chr13:19669218-19669677	-4371	inter	Treg	no	LCR	yes	DHS	Treg Mono/Di
<i>MAPK11P1L</i> 93487	0.03	0.23	A_24_P107674	chr13:197643111-19764922	-99540	inter	Treg		ICR	no	no	no H3K4me
<i>LGALS3</i> 3958	3.85	2.31	A_23_P128919	chr14:54608917-54609194	20941	inter	Treg		LCR	no	DHS	ND
<i>NOG</i> 9241	-1.08	5.52	A_23_P341938	chr14:54670099-54670231	4435	intra	Tconv		LCR	no	no	Treg Mono/Di
<i>SEPT9</i> 10801	-0.78	0.66	A_24_P147910	chr17:52024396-52024835	-1443	inter	Tconv	no	LCR	no	DHS	Treg Mono/Di
<i>EPS15L1</i> 10365	-0.74	-0.74	A_23_P38941	chr17:72787432-72787562	-1626	inter	Treg		LCR	no	no	no H3K4me
<i>IL2RB</i> 3560	1.63	0.62	A_24_P203000	chr17:72882994-72883638	-442	5-proximal	Tconv	yes	LCR	yes	no	Tconv Tri
<i>CACNA1F</i> 778	-0.27	-0.09	A_23_P148327	chr17:72914128-72914221	1422	intra	Treg		LCR	no	DHS	Treg Tri
<i>FOXP3</i> 7575	3.68	4.60	A_23_P159709	chr19:16323001-16323171	-3971	inter	Treg		LCR	no	DHS	Treg Mono/Di
<i>PPP1R3F</i> 89801	0.21	1.59	A_24_P177604	chr22:35871285-35872166	4176	intra	Treg	yes	LCR	no	no	Treg Tri
<i>CD40LG</i> 959	-2.48	-4.70	A_23_P62220	chr22:35893803-35894363	-18181	intra	Treg	yes	LCR	no	no	Treg Tri
				chrX:48975534-48975593	1180	intra	Treg		LCR	no	no	ND
				chrX:49002019-49005207	4581	intra	Treg	yes	LCR	yes	no	Treg Tri
				chrX:49007147-49009512	-136	5-proximal	Treg	yes	ICR	yes	no	Treg Tri
				chrX:49020095-49022146	7093	intra	Treg	yes	LCR	yes	DHS	Treg Tri
				chrX:49022670-49023240	8927	intra	Treg	yes	LCR	no	no	Treg Tri
				chrX:135561484-135561563	3517	intra	Tconv		LCR	no	DHS	Tconv Tri
				chrX:135566710-135566897	8797	intra	Tconv	yes	LCR	yes	no	Tconv Tri

^a Coloring indicates larger than 2 fold differences between gene expression levels as determined by microarray analysis. Genes with higher expression in Treg cells are marked in red, those with higher expression in Tconv cells in blue.

^b DMR were classified either as intergenic (inter) or intragenic (intra) based on their position relative to known genes.

^c DMR were classified according to their CpG content using the promoter definitions proposed by Weber et al. (2005) as described in the Methods section.

^d Overlap of the identified DMR with conserved sequence elements predicted by the phastCons program based on a whole-genome alignment of vertebrates was done using the Table Browser at the UCSC Genome Browser web site (genome.ucsc.edu/).

^e Overlap of the identified DMR with DNaseI hypersensitive sites (DHS) in CD4⁺ T-cells (Boyle et al, 2008) was determined using the Table Browser at the UCSC Genome Browser web site (genome.ucsc.edu/)

^f Histone H3 lysine 4 methylation at DMR regions was classified as follows: Treg or Tconv Tri, increased H3K4 trimethylation in Treg or Tconv cells, respectively; Treg or Tconv Mono/Di, increased H3K4 mono-, or di-, but no trimethylation in Treg or Tconv cells, respectively; ND, H3K4 methylation present but not different between T cell subsets.

Supplemental Table S3
Sequence motifs associated with DMR

DMR class	TF Matrices	Number of Sequences ^a	Number of Matches ^b	Expected (genome) ^c	Std.dev.	Over representation (genome) ^d	Z-Score (genome) ^e
DMR hypomethylated in Tconv cells (n=31)	V\$ATF.01	10	11	2.35	1.53	4.68	5.32
	V\$CREB.02	9	11	2.42	1.55	4.55	5.2
	V\$CTCF.03	6	13	3.7	1.92	3.51	4.57
	V\$AHRARNT.03	9	9	2.27	1.51	3.96	4.13
	V\$ELK1.02	8	10	2.98	1.73	3.36	3.78
	V\$ATF6.02	8	9	2.52	1.59	3.57	3.77
	V\$AP1.03	8	14	5.21	2.28	2.69	3.63
	V\$NUDR.01	8	8	2.32	1.52	3.45	3.4
	V\$ATF.02	9	13	5.21	2.28	2.5	3.2
	V\$CTCF.04	4	9	3.03	1.74	2.97	3.14
	V\$ZF5.01	3	7	2.08	1.44	3.37	3.07
	V\$XBP1.01	6	9	3.16	1.78	2.85	3
DMR hypomethylated in Treg cells (n=101)	V\$NRF1.01	4	21	4.96	2.23	4.23	6.98
	V\$ZF5.01	9	24	6.77	2.6	3.55	6.43
	V\$VMYB.05	32	35	15.56	3.94	2.25	4.8
	V\$STAT5.01	30	66	36.75	6.06	1.8	4.75
	V\$VMYB.03	30	33	15.51	3.94	2.13	4.31
	V\$HMX2.01	28	55	30.92	5.56	1.78	4.24
	V\$ZNF219.01	17	36	18.47	4.3	1.95	3.96
	V\$KKLF.01	25	38	20.2	4.49	1.88	3.85
	V\$VMYB.04	32	38	20.78	4.56	1.83	3.67
	V\$MZF1.03	36	42	23.92	4.89	1.76	3.6
	V\$STAT.01	47	75	49.27	7.02	1.52	3.6
	V\$MZF1.01	37	43	24.7	4.97	1.74	3.58
	V\$NFKAPPAB65.01	28	36	20.05	4.48	1.8	3.45
	V\$MAZR.01	18	31	16.84	4.1	1.84	3.33
	V\$OLF1.01	20	27	14.14	3.76	1.91	3.29
	V\$VMYB.02	26	30	16.36	4.04	1.83	3.25
	V\$MZF1.02	43	52	32.93	5.74	1.58	3.24
	V\$HIVEP1.01	22	32	17.93	4.23	1.78	3.21
	V\$FLI.01	27	27	14.47	3.8	1.87	3.16
	V\$ELK1.02	19	20	9.7	3.11	2.06	3.15
	V\$HMX2.02	30	68	46.41	6.81	1.47	3.1
	V\$E2F.01	36	45	28.51	5.34	1.58	3

^a Number of input sequences with at least one match.

^b Number of matches in all sequences.

^c Expected match numbers in an equally sized sample of the genomic background and the standard deviation.

^d Overrepresentation against genomic background: Fold factor of match numbers in regions compared to an equally sized sample of the background (i.e. found versus expected).

^e Z-score of overrepresentation against genomic background: The distance from the population mean in units of the population standard deviation. Shown are only highly significant motifs (Z score >3; a Z-score above 2 can be considered statistically significant).

Supplemental Table S4

Oligonucleotides for bisulfite amplicon generation

Amplicon	Chromosomal Location (hg18)	Sense	Antisense	Dir.
Epi00197_TNFRSF9.1	chr1:7932138-7932511	aggaagagagGGGTGTAGGTGATAATTGTGATTAAA	cagtaatacgactcactataggagaaggctCTTCCATTATAATAAAACACAAAAAACA	+
Epi00003_IL2RA.1	chr10:6118877-6119370	aggaagagagTTGTAGATTGGGATTGTTAGGGTA	cagtaatacgactcactataggagaaggctCTAAATTCACCCAAAAACAAAAAA	+
Epi00004_IL2RA.2	chr10:6119558-6119803	aggaagagagAGAGTTTGGGTTATTGGGTAAGAG	cagtaatacgactcactataggagaaggctCCACAAAAATTTCTCTAAAAATCA	+
Epi00005_IL2RA.3	chr10:6120520-6121011	aggaagagagATAGTTTAAGGTGGTGGGATAGGAG	cagtaatacgactcactataggagaaggctCAATCCAACATTCTATAACTACAAAATTA	+
Epi00203_IL2RA.1	chr10:6121399-6121972	aggaagagagTTTTTTTATGATGGATAGGATAGATAGA	cagtaatacgactcactataggagaaggctTTTTACACATTCTCTACCAAAATAACC	+
Epi00006_IL2RA.4	chr10:6122010-6122464	aggaagagagTTATAGGTAGAATGTTTTGTGAAGTATGA	cagtaatacgactcactataggagaaggctTAAACAAACAACCATCAAAAAT	+
Epi00017_IL2RA.15	chr10:6153564-6153844	aggaagagagGTAGTTTTTGGGGTAATATTGAGG	cagtaatacgactcactataggagaaggctAAACAAAAATTCATCCAATACCAA	+
Epi00018_IL2RA.16	chr10:6153808-6154224	aggaagagagATATTGGTTTGATTGGTATTGGATG	cagtaatacgactcactataggagaaggctAAAAATCCTACCACCTCAACCTACT	+
Epi00019_IL2RA.17	chr10:6154058-6154547	aggaagagagTATTTTGGGAAGTTAAGGTAGGAGG	cagtaatacgactcactataggagaaggctTTCATTACCCAAAAATCCCTACTT	+
Epi00020_IL2RA.18	chr10:6154524-6154923	aggaagagagAGTAGGGATTTTTTGGGTAATGAAG	cagtaatacgactcactataggagaaggctAAAAAACTAAAAATTCATCCACAC	+
Epi00021_IL2RA.19	chr10:6155117-6155433	aggaagagagTTTTTTTAGTTATTTTGGGTTTTG	cagtaatacgactcactataggagaaggctAACTCACACTTATAATCCCAACACTTT	+
lrrc32_6534	chr11:76030804-76031729	aggaagagagTTTATAGTTGGTTGGGATGTAGATAATG	cagtaatacgactcactataggagaaggctCACCAAACTATCAACCTTCAAAAA	+
Epi00205_LRRC32.1	chr11:76055736-76056113	aggaagagagTTTTGAGTTTTAGTTTTTTATTGAGG	cagtaatacgactcactataggagaaggctAAATACCTTTTCTCTACAACATCC	+
Epi00206_LRRC32.2	chr11:76056033-76056454	aggaagagagTTTTAGGTTTTTATAGTGGGTGTTTT	cagtaatacgactcactataggagaaggctACTAACCACAAACATACTCCCC	+
Epi00207_LRRC32.3	chr11:76056414-76056952	aggaagagagGGTTTTTAGGTTATTGGGGAGTAT	cagtaatacgactcactataggagaaggctCAAAAAACAAATCAAAACCCACTA	+
Epi00026_IFNG.1	chr12:66844056-66844260	aggaagagagATGGTTAGAAGGTATAAAAGAAAAGGG	cagtaatacgactcactataggagaaggctAAATCAATATTAATCCATACCCCC	-
il26_6531	chr12:66878511-66879260	aggaagagagTTTGATTAGGGTTGAGGGAGAAG	cagtaatacgactcactataggagaaggctCCCACAAATACCAATTTAAAAAAA	+
Epi00208_GJB2.1	chr13:19668983-19669485	aggaagagagTGTTAAAAAGTATATTTGGTTAGAAATGA	cagtaatacgactcactataggagaaggctTAAACCACAACTCCATATCCAAT	+
Epi00209_GJB2.2	chr13:19669465-19670046	aggaagagagGATATGGAGTTTGTGGTTTTAGAAATTT	cagtaatacgactcactataggagaaggctCAACACCATTTACATAAAATAACAA	+
Epi00210_GJB2.3	chr13:19669565-19670072	aggaagagagGAAAGAAGTTTTTGTGTTTTTTGAT	cagtaatacgactcactataggagaaggctAACCTACTATCTCTCTCTTAATAACAA	+
Epi00027_NOG.1	chr17:52016154-52016611	aggaagagagTTTTTTTGGGTTAGGGTTTGAAAG	cagtaatacgactcactataggagaaggctACTTAACCTCTTTTCCCTTCCCT	+
Epi00030_NOG.4	chr17:52024346-52024855	aggaagagagTGGAAGATTGGTAAATATTGAGTT	cagtaatacgactcactataggagaaggctAAAATCTCCAAACCCCAATATA	+
Epi00211_SEPT9.1	chr17:72882880-72883313	aggaagagagTTAGGGGTTTTTTTGTGTTTTTAAATGT	cagtaatacgactcactataggagaaggctCACCAAAATAATCTCAATAAACCCA	+
Epi00212_SEPT9.2	chr17:72883369-72883770	aggaagagagGGTTTTAATTTGTTGGTTTTTTGTG	cagtaatacgactcactataggagaaggctTCCATACTAATTCTCCCCTACTAATA	+
Epi00241_ID2.1	chr2:8726166-8726763	aggaagagagTGGATGGATGTTTTAAAGTTTAGTTATT	cagtaatacgactcactataggagaaggctAAACCACCATCATATTTAACAACATTA	+
Epi00047_ID2.1	chr2:8734408-8734815	aggaagagagTGTGTTTTTTGTTAGGGATTGTAAGT	cagtaatacgactcactataggagaaggctCTTTCACAAAAATTTTCTATATCTT	+
Epi00048_ID2.2	chr2:8735298-8735692	aggaagagagATTTGGTTTTAGGGTAAGGGTTTTT	cagtaatacgactcactataggagaaggctAAACCAAAACTTCCAATCAACTT	+
Epi00049_ID2.3	chr2:8735719-8736002	aggaagagagTATTAGAAAGGGGATTGTTTGGTT	cagtaatacgactcactataggagaaggctAACTTTAATCCTAAATTCCTAAAAATACC	+
Epi00050_ID2.4	chr2:8736649-8736919	aggaagagagGGAATGGATATAGTTGTGAGAATAAAA	cagtaatacgactcactataggagaaggctCAACCTAATCCAAAACCTCACTCAC	+
Epi00051_ID2.5	chr2:8738550-8738834	aggaagagagAGTTTTGGAATTTTTTTAGGTGTTG	cagtaatacgactcactataggagaaggctAAATACTTATTACAAACCATACCAACC	+
Epi00052_ID2.6	chr2:8738655-8739088	aggaagagagGTTTTTTAAGGGTAGTGATGTAATG	cagtaatacgactcactataggagaaggctAAAAAAACCACAATTCACTACAACC	+
Epi00053_ID2.7	chr2:8739870-8740348	aggaagagagTGTTATTTTAAGTTTAAGGAGTTGGTGT	cagtaatacgactcactataggagaaggctTTTTTATAATCCACAAACCAACAAA	+
Epi00054_ID2.8	chr2:8740456-8740944	aggaagagagTGATAGTAAAGTATTGTGTGGTTGAA	cagtaatacgactcactataggagaaggctAAAATCCCACATCACAAAATTAATA	+
Epi00055_ID2.9	chr2:8741022-8741291	aggaagagagTTGTTGTTGGAGATTTAAATAGGAGA	cagtaatacgactcactataggagaaggctCAAAAAATAAAAAAATCATAAACACCTAC	+
Epi00246_ID2.6	chr2:8748975-8749253	aggaagagagGGTTGTTAATAAAGAAATGATTATTTGAA	cagtaatacgactcactataggagaaggctTTAAACCAATTATCCAAAAATACCC	+
Epi00032_CTLA4.1	chr2:204407956-204408452	aggaagagagGTGTTTATGTGAGTTGAGGGATTAT	cagtaatacgactcactataggagaaggctCAATCCAATTACAACCATAAAAATA	+
Epi00033_CTLA4.2	chr2:204408649-204408984	aggaagagagTGTTGTTGTTGGTTGTAAGTATTGTT	cagtaatacgactcactataggagaaggctACCTACCCACTTACTCTAATTCTCA	+
Epi00213_CTLA4.1	chr2:204409089-204409645	aggaagagagTTTTTTGTTGTGATATTGTTTTAGG	cagtaatacgactcactataggagaaggctATCCCTTCTAACCATTCAAATTTCT	+
Epi00034_CTLA4.3	chr2:204409465-204409854	aggaagagagGGTATTGGAGTTATTGAGTTGGTAGA	cagtaatacgactcactataggagaaggctCCCCTACATACAAAAAACAACATA	+
Epi00214_CTLA4.2	chr2:204410553-204411141	aggaagagagTGAGTGAAAGAAGTTTATATGAAAAGGT	cagtaatacgactcactataggagaaggctTCATTTTCATAACATATAACATCAATCAA	+
Epi00216_CTLA4.4	chr2:204426356-204426766	aggaagagagTTAAGTTTTAATTGGGTTAGGTTTG	cagtaatacgactcactataggagaaggctTACCAACATCTAAAAACATCAA	+

Supplemental Table S4

Oligonucleotides for bisulfite amplicon generation (continued)

Amplicon	Chromosomal Location (hg18)	Sense	Antisense	Dir.
Epi00217_CTLA4.5	chr2:204426643-204427110	aggaagagagTATGGAATTTTTATGTGAGGTTTGT	cagtaatacgactcactataggagaaggctAACCAAAACAAAACCTCAATCCTCT	+
Epi00038_CTLA4.7	chr2:204427109-204427432	aggaagagagTTTTTTTGGTTGTTTGTGTTTGATT	cagtaatacgactcactataggagaaggctAAAAAAATTTCTCTTACCTACC	+
Epi00219_CTLA4.7	chr2:204429550-204430103	aggaagagagTTATGATTAAATTTAGTGTGATTAAATTGGA	cagtaatacgactcactataggagaaggctAAATAAACTTTCTAAAATTCCTCAA	+
Epi00220_CTLA4.8	chr2:204430849-204431377	aggaagagagTTTTGTATGTGGTAAGAATTTATGTGA	cagtaatacgactcactataggagaaggctCCATCACCACCAAAATCTAAAATA	+
Epi00221_CTLA4.9	chr2:204431356-204431929	aggaagagagTTTAGATTTTGGTTGGTGATGG	cagtaatacgactcactataggagaaggctAAAATAACCAACACAAAAAACAC	+
Epi00222_CTLA4.10	chr2:204432444-204433038	aggaagagagTTTAGGAGTTAGTGTGTTGTATAGATTGTG	cagtaatacgactcactataggagaaggctCCCCACCTAAATAATACATTCAAAA	+
Epi00226_CTLA4.14	chr2:204441638-204442166	aggaagagagAAGGAAAAGGAAAGAAAGAAAGTTATTA	cagtaatacgactcactataggagaaggctTTATAACCCACCCAAATAAACACTC	+
Epi00227_CTLA4.15	chr2:204443006-204443543	aggaagagagTGGGTGATAGAGGTTTAGGGTTAGT	cagtaatacgactcactataggagaaggctAAAACCAAAAAAACTCAATAAACTCA	+
Epi00233_CTLA4.21	chr2:204445693-204446278	aggaagagagTTTTATAATAGGGGTTTATGTGAAAATG	cagtaatacgactcactataggagaaggctCCTTTAACATCACTAACTAAACATAACCA	+
Epi00234_CTLA4.22	chr2:204446252-204446789	aggaagagagTTATGTTTTAGTTAGTGATGTTAAAGGTTG	cagtaatacgactcactataggagaaggctTCTTCTATCCATAACATTAACCACATATT	+
Epi00236_IKZF2.2	chr2:213697351-213697584	aggaagagagTGAAATTGTTATTGTGTAGAAGGGG	cagtaatacgactcactataggagaaggctCAAAAAAATTCATTACATAACATATCCA	+
Epi00040_ZNFN1A2.1	chr2:213723180-213723764	aggaagagagTTTTTTAATTTTTAAAGAGGAGGTGA	cagtaatacgactcactataggagaaggctTTACCAAAAACCAAAAAAATCCTTA	+
Epi00041_ZNFN1A2.2	chr2:213723485-213723925	aggaagagagTTTTTAGGGATGGTTTAGTAGGAAAA	cagtaatacgactcactataggagaaggctTCCAAATAAAAAATAATATCCAAATCC	+
Epi00239_IKZF2.5	chr2:213726239-213726736	aggaagagagAGAGAAATTTTGGGTTTAGGTTTGTA	cagtaatacgactcactataggagaaggctAACCAAAAACAAAACCTACATCAAC	+
Epi00045_ZNFA1N2.2	chr2:213727375-213727718	aggaagagagAGGGTTTTATTATGTTGGTTAGGGT	cagtaatacgactcactataggagaaggctAACTAAAAATCTATTTCTCTCCCA	+
Epi00046_ZNFN1A2.1	chr2:213809715-213810256	aggaagagagGGGAAGGTAGTATTATTTTTGTTTTT	cagtaatacgactcactataggagaaggctAATCTCTCTAAATTCATTAATAATCA	+
Epi00240_WNT10A.1	chr2:219476834-219477390	aggaagagagAGTTTTTTAAAGTGTGGGATTATAGG	cagtaatacgactcactataggagaaggctAAACCCAAATTAATACAAAAATCCA	+
Epi00248_IL2RB.2	chr22:35871983-35872251	aggaagagagTTTTTATAGGGGATGTTTTTGAT	cagtaatacgactcactataggagaaggctCAAAAAAACAAATAAAACCTACA	+
Epi00251_IL2RB.5	chr22:35893508-35893730	aggaagagagGTTTTATTGTTTTGGTTGTTTGGT	cagtaatacgactcactataggagaaggctAAACAAATCTCCACCTATACC	+
Epi00253_PDE4D.1	chr5:58457591-58458087	aggaagagagGGGGTTTTGATTTTTGTGATATAATTT	cagtaatacgactcactataggagaaggctAATAACTAAAAACAACTTCTCTCTTTC	+
Epi00254_PDE4D.2	chr5:58458185-58458681	aggaagagagGGGTGTTTTGTTTTAGTATTAGTTTTATTGA	cagtaatacgactcactataggagaaggctATTTCAACTTTCACAAACACTCCA	+
Epi00067_TNF.1	chr6:31650965-31651237	aggaagagagTTTGGTTTTTAAAGAAATGGAGGT	cagtaatacgactcactataggagaaggctTCCTTAATAAAAAACCCATAAACTCA	+
Epi00068_TNF.2	chr6:31651106-31651692	aggaagagagGAGGATTTTTTGTGTTTGTGTTT	cagtaatacgactcactataggagaaggctAACACTCACCTCTTCCCTCTAAAAA	+
Epi00069_TNF.3	chr6:31651639-31652152	aggaagagagTTTTGTTTGTGATTTTGGAGTGA	cagtaatacgactcactataggagaaggctAAACATTCAACAACTCTTCCCTAA	+
Epi00070_TNF.4	chr6:31651639-31651905	aggaagagagTTTTGTTTGTGATTTTGGAGTGA	cagtaatacgactcactataggagaaggctAAACACCTTCCATATACCAACATC	+
Epi00071_TNF.5	chr6:31652127-31652504	aggaagagagTTTAGGAAAGAGTTGTTGAATGTT	cagtaatacgactcactataggagaaggctAAAAAAACTAAAACCTTAAACTTCC	+
Epi00074_HOXA1.1	chr7:27095653-27096004	aggaagagagGGTTTAGAGTTAGAATTTTTTTGGAA	cagtaatacgactcactataggagaaggctTTACTAACCCCACTCAATCAAAT	+
Epi00075_HOXA1.2	chr7:27096226-27096699	aggaagagagGAGATTAGGGATGTGGGTTTTATTTA	cagtaatacgactcactataggagaaggctCTCTTCAAAAATCAAATTCATATAATCA	+
Epi00077_GATA4.1	chr8:11583609-11584063	aggaagagagTATATTGAGGAGGTGGTTTTGTTTT	cagtaatacgactcactataggagaaggctTTAACCCATAAAAAATTCAAAAATC	+
Epi00259_TP53INP1.1	chr8:96010388-96010962	aggaagagagTAGAGGAAATTAGTTAGAGTGGATGGT	cagtaatacgactcactataggagaaggctTCCACAAAATAAAAAATCTCTATCAT	+
Epi00269_FOXP3.1	chrX:48996840-48997010	aggaagagagATTAAAGGATGTAAGAGGTTAAATGGT	cagtaatacgactcactataggagaaggctAATAATTCAAAAAACCTCTCTTTC	+
Epi00270_FOXP3.2	chrX:48997485-48998052	aggaagagagGAGAGGTTGGTGATTTAGAGGTTTA	cagtaatacgactcactataggagaaggctCCAAAAAATTTAAATACTTTCCCA	+
Epi00271_FOXP3.3	chrX:48997580-48997983	aggaagagagAGGTTGGAGTGTAGTGGTGAATTT	cagtaatacgactcactataggagaaggctATAATCCCAACATCAATAACCACAT	+
Epi00272_FOXP3.4	chrX:49001667-49002039	aggaagagagGTTTGTGTTTTTTGGGTTTAGGGT	cagtaatacgactcactataggagaaggctATCCCAACCAATCTACTTTAACC	+
Epi00081_FOXP3.1	chrX:49003029-49003414	aggaagagagGGGTTTTTTGTGATTTTAGAATTT	cagtaatacgactcactataggagaaggctCTAACCAACCCCAAAAAATAAC	+
Epi00082_FOXP3.2	chrX:49003993-49004485	aggaagagagATTTGTTTGGGGTAGAGGATTAG	cagtaatacgactcactataggagaaggctACCCAAAAATCCCAATATCTATAA	+
Epi00083_FOXP3.3	chrX:49004673-49005149	aggaagagagAGGGTTTTTTGTTTATTAGGTTTGG	cagtaatacgactcactataggagaaggctAAAAAAACCTAAACTACCATTCCC	+
Epi00088_FOXP3.8	chrX:49005022-49005472	aggaagagagTTTTTGTGTGTGTTTTTTGTTTTT	cagtaatacgactcactataggagaaggctAAACCTCACCTAACCAACTCTTAT	+
Epi00084_FOXP3.4	chrX:49005180-49005620	aggaagagagGGTTTTTAGTTGGGAGAGAGTTAG	cagtaatacgactcactataggagaaggctTTTCAACAACATCAATTAACCAA	+
Epi00277_FOXP3.9	chrX:49006709-49007185	aggaagagagTTTAGGGTTAGTTTAAGTAGAGGGAGT	cagtaatacgactcactataggagaaggctAACCAAAATCCATATCAAAAAACA	+
Epi00278_FOXP3.10	chrX:49007665-49008089	aggaagagagTAGAGAGATAGAGAAGGATGAGAGGTATT	cagtaatacgactcactataggagaaggctTTCTATCAATCCACTTCACCAAAAT	+

Supplemental Table S4

Oligonucleotides for bisulfite amplicon generation (continued)

Amplicon	Chromosomal Location (hg18)	Sense	Antisense	Dir.
Epi00090_FOXP3.10	chrX:49008021-49008450	aggaagagagAAAAATTGTGGTTTTTTATGAGTTT	cagtaatacgactcactatagggagaaggctCCTCCAATAAAACCCACATCTAATA	-
Epi00281_FOXP3.13	chrX:49008533-49009058	aggaagagagAGAGATATTATTTGTGAGTGAGAGGA	cagtaatacgactcactatagggagaaggctTAAACAAAAAATCAAAATCCCAA	+
Epi00282_FOXP3.14	chrX:49009597-49010138	aggaagagagTTTTTTTGATAGGTTATGGTGAAGA	cagtaatacgactcactatagggagaaggctCTACAAACCTCCAAACAAAAAACCT	+
Epi00285_FOXP3.17	chrX:49018325-49018803	aggaagagagTTTTTTTGTGTTTTGGGTTTTAGTT	cagtaatacgactcactatagggagaaggctTTAAACCATCAATCAAATAAAAAATACC	+
Epi00286_FOXP3.18	chrX:49019307-49019834	aggaagagagTATTTATAATTGAAGGGATGGGGAT	cagtaatacgactcactatagggagaaggctCCACCTATCAACTATATAACCTAAAACAA	+
Epi00091_FOXP3.11	chrX:49020173-49020505	aggaagagagAGTTGAGGTTGATATATTTGTTGGG	cagtaatacgactcactatagggagaaggctAATCACAACCTACCATTACCACCAC	+
Epi00092_FOXP3.12	chrX:49021142-49021481	aggaagagagTTTAAAGTGGAGTTTTTTTGGAGTG	cagtaatacgactcactatagggagaaggctCAAAACACCTTTCCATAACTAAAA	+
Epi00289_FOXP3.21	chrX:49022000-49022496	aggaagagagTTGGTGGGATTTGTGAGTTTATAGATA	cagtaatacgactcactatagggagaaggctCCTCACAATCACCTATAAAACAAA	+
Epi00290_FOXP3.22	chrX:49022492-49022978	aggaagagagTGAGGATTGAATTAATATATGTTGTTAGG	cagtaatacgactcactatagggagaaggctTCAAAAATTACAAATACCCACCATC	+
Epi00261_CD40LG.1	chrX:135566523-135567022	aggaagagagTTTTGATAATATAGAAGAAATGGTATGTAG	cagtaatacgactcactatagggagaaggctACACACACACATACACTACTTAAACTC	+
Epi00262_CD40LG.2	chrX:135567084-135567502	aggaagagagGTTTTATTTTGAAGTGGTTTGGGT	cagtaatacgactcactatagggagaaggctTCTCTATTATTATCATCTAATCAAAAAA	+
Epi00263_CD40LG.3	chrX:135568170-135568667	aggaagagagTTGGTGGGTATTTTGGTTTAGTTAT	cagtaatacgactcactatagggagaaggctCCATAAACTTCAATTCCTCATCTACA	+
Epi00265_CD40LG.5	chrX:135568910-135569144	aggaagagagGGTAATTTTGGAAAATGGGAAATAG	cagtaatacgactcactatagggagaaggctTTCAAATACTCCTCCCAAATAAATAA	+

Supplemental Table S6

Oligonucleotides for the amplification of DMR regions

Symbol	Chromosomal Location (hg18)	Sense primer	Antisense primer	Cloning
<i>CTLA4</i>	chr2:204408509-204409748	ATTAAAAGGAATTCCTGCAGTTTCTCAGTGCCTACAAGGTG	GCTCTTCTCCACTAGTTTGTCCAAATTCATCCCTACTCC	inFusion
	chr2:204426486-204427513	ATTAAAAGGAATTCCTGCAGAACCACGGTCTAGTTTCAACC	GCTCTTCTCCACTAGTTTGTGAGAATTGAGGAGAAGGC	inFusion
<i>FOXP3</i>	chrX:49003778-49004624	ATTAAAAGGAATTCCTGCAGGCCAGAACAACTAATACCGA	GCTCTTCTCCACTAGTCTACACATACTGAGACTTTGGG	inFusion
	chrX:49018113-49019122	ATTAAAAGGAATTCCTGCAGGAATGAAAGCAGACCATGTCC	GCTCTTCTCCACTAGTTTGATCCACTCGTTTCTCCTC	inFusion
	chrX:49020216-49021443	ATTAAAAGGAATTCCTGCAGTTGAGCCAATCCCAAGATCC	GCTCTTCTCCACTAGTTTTCACAGCCACCTTCAAACC	inFusion
<i>IKZF2</i>	chr2:213809332-213810300	ATTAAAAGGAATTCCTGCAGGACTCTATCAGATACTTTCAGTGTGG	GCTCTTCTCCACTAGTATTCTCATTCTGTTCACTTCACCTC	inFusion
<i>IL1R1</i>	chr2:102045190-102046401	ATTAAAAGGAATTCCTGCAGATTCTTTGTAACCTGGCTGAC	GCTCTTCTCCACTAGTGTGGATTCTGACTCTGGTGG	inFusion
<i>IL2RA</i>	chr10:6153878-6154935	ATTAAAAGGAATTCCTGCAGTAGAAATTCATCCACACCCAC	GCTCTTCTCCACTAGTGCTGAGTAGGAGAGGAAGAC	inFusion
	chr10:6118859-6120070	ATTAAAAGGAATTCCTGCAGGCAGACTGGGATTTGTGAGG	GCTCTTCTCCACTAGTGGTTGGCCTAAATGATCTTTGAG	inFusion
	chr10:6121540-6122683	ATTAAAAGGAATTCCTGCAGGCAAGAGTGGGTATCTATGG	GCTCTTCTCCACTAGTCAAAGATGCCATTAAGTCCTTGAG	inFusion
<i>IL2RB</i>	chr22:35871120-35872336	ATTAAAAGGAATTCCTGCAGTAGGAGCTCTGACCCAAACAC	GCTCTTCTCCACTAGTTTAGGCTTTGATTGCAACAGG	inFusion
	chr22:35893420-35894563	ATTAAAAGGAATTCCTGCAGGTCGCATGTTTCAGATGCAG	GCTCTTCTCCACTAGTCTTATGCAGTATCCAGGCCTC	inFusion
<i>LGALS3</i>	chr14:54608337-54609182	ATTAAAAGGAATTCCTGCAGGCAGTTACCAGTCATTGGAG	GCTCTTCTCCACTAGTCTATATTAGATGTGCATTGTTTGGG	inFusion
	chr14:54639182-54640281	ATTAAAAGGAATTCCTGCAGATACACCTTCCCTAAGCAATTCC	GCTCTTCTCCACTAGTATCTAAACCACTCATGCCCTG	inFusion
<i>LRRC32</i>	chr11:76055840-76056973	ATTAAAAGGAATTCCTGCAGGGCATGAACCTATTCCATCC	GCTCTTCTCCACTAGTTCTTTTCAGGGAGAGCAGTCAG	inFusion
	chr11:76030698-76031632	ATTAAAAGGAATTCCTGCAGGGCTACACTATCAAGGGAAGG	GCTCTTCTCCACTAGTTTGAACCTAGACTTTGATGCTCATGG	inFusion
<i>TP53INP1</i>	chr8:96009677-96010763	ATTAAAAGGAATTCCTGCAGAATCTCATGGCTGAACCTCTG	GCTCTTCTCCACTAGTTCTTTAACCACTCTGGCCTC	inFusion
<i>CD40LG</i>	chrX:135566244-135567359	GATGAATGCATGTACAGCACTCGACAGCATCAC	ATCCTACTAGTAGCATCTGAGCGGTAGCCAC	
<i>CHD7</i>	chr8:61987563-61988493	GATGAATGCATCCTGTTCTCACCTGCATCTGGG	ATCCTACTAGTGCAAGTGCTCTTGGTCTCTGTC	
	chr8:61755811-61756623	GATGAATGCATAAATGTCTCCTACTTATCACCTAGAAAGGC	ATCCTACTAGTCCCTCTTCTGAACAGACAATGGAC	
<i>ID2</i>	chr2:8737174-8738191	GATGAATGCATTAAAGGTGATTTTCATATTGGCGTGCTG	ATCCTGCTAGCTTTGCATTTTCAGAAAGCGGAAGGG	
<i>IFNG</i>	chr12:66843471-66844447	GATGAATGCATTCAATCGAAGTATTTGGCACTTGGT	ATCCTACTAGTCAGACTGGCAGTGAACATCTGCT	
<i>IL26</i>	chr12:66878596-66879204	GATGAATGCATGTGCTTCTGACTTTGATTCTCCA	ATCCTACTAGTCATCTGAACAATCCACAAGCCTCAC	
<i>SEPT9</i>	chr17:72882782-72883825	GATGAATGCATTGTGTTCCGTTGAGCCTCCTG	ATCCTACTAGTTCTCTTGCAATCCAATTCTACTCTCC	

Supplemental Table S7
Properties of DMR analyzed by enhancer reporter assays in Jurkat T cells

Closest Gene (Symbol) ^a	Chromosomal Location (hg18)	Hypomethylated T cell type	Overlap with PhastCons ^b	Overlap with DHS in CD4 ⁺ T cells ^c	Relative Position to Gene Transcripts ^d	CpG content class ^e	H3K4 Methylation Status ^f	Epityper validation of MCIP results	Repotergene Active in Jurkat Cells
<i>IFNG</i>	chr12:66843471-66844447	Tconv	yes		inter	LCR	Tconv Mono/Di	yes	yes
<i>IL26</i>	chr12:66878596-66879204	Tconv	yes	DHS	inter	ICR	ND	yes	yes
<i>SEPT9</i>	chr17:72882782-72883825	Tconv	yes		intra	LCR	Tconv Tri	yes	yes
<i>ID2</i>	chr2:8737174-8738191	Tconv	yes	DHS	inter	LCR	Tconv Tri		yes
<i>CHD7</i>	chr8:61755811-61756623	Tconv		DHS	intra	ICR	Tconv Tri		
<i>CHD7</i>	chr8:61987563-61988493	Tconv	yes	DHS	inter	LCR	Tconv Mono/Di		
<i>CD40LG</i>	chrX:135566244-135567359	Tconv	yes		intra	LCR	Tconv Tri	yes	yes
<i>IL2RA</i>	chr10:6118859-6120070	Treg		DHS	intra	LCR	Treg Tri	yes	
<i>IL2RA</i>	chr10:6121540-6122683	Treg			intra	LCR	Treg Tri	yes	yes
<i>IL2RA</i>	chr10:6153878-6154935	Treg		DHS	inter	LCR	Treg Tri	yes	
<i>LRRC32</i>	chr11:76030698-76031632	Treg	yes		inter	LCR	Treg Tri	yes	yes
<i>LRRC32</i>	chr11:76055840-76056973	Treg			intra	LCR	Treg Tri	yes	yes
<i>MAPK1IP1L</i>	chr14:54608337-54609182	Treg		DHS	inter	LCR	ND		
<i>LGALS3</i>	chr14:54639182-54640281	Treg			inter	LCR	ND		yes
<i>IL1R2</i>	chr2:102045190-102046401	Treg			inter	LCR	ND		
<i>CTLA4</i>	chr2:204408509-204409748	Treg	yes		inter	LCR	Treg Tri	yes	
<i>CTLA4</i>	chr2:204426486-204427513	Treg	yes	DHS	inter	LCR	Treg Tri	yes	
<i>SPAG16</i>	chr2:213809332-213810300	Treg	yes		inter	LCR	Treg Tri	yes	
<i>IL2RB</i>	chr22:35871120-35872336	Treg			intra	LCR	Treg Tri	yes	
<i>IL2RB</i>	chr22:35893420-35894563	Treg			intra	LCR	Treg Tri	yes	
<i>TP53INP1</i>	chr8:96009677-96010763	Treg	yes	DHS	intra	LCR	Treg Tri	yes	yes
<i>FOXP3</i>	chrX:49003778-49004624	Treg	yes		intra	LCR	Treg Tri	yes	yes
<i>PPP1R3F</i>	chrX:49018113-49019122	Treg			intra	LCR	Treg Tri	yes	
<i>PPP1R3F</i>	chrX:49020216-49021443	Treg	yes	DHS	intra	LCR	Treg Tri	yes	yes

^a For reporter gene assays, we preferentially selected differentially methylated regions (DMR) that were associated with genes that also showed differential gene expression. Shown are genes with the smallest distance between TSS and DMR. *MAPK1IP1L* and *SPAG16* were not differentially expressed in T cell subsets, however, the corresponding genes in the opposite direction (*LGALS* and *IKZF2*) were specifically expressed in Treg cells.

^b Overlap of the identified DMR with conserved sequence elements predicted by the phastCons program based on a whole-genome alignment of vertebrates was done using the Table Browser at the UCSC Genome Browser web site (genome.ucsc.edu/).

^c Overlap of the identified DMR with DNaseI hypersensitive sites (DHS) in CD4⁺ T-cells (Boyle et al, 2008) was determined using the Table Browser at the UCSC Genome Browser web site (genome.ucsc.edu/).

^d DMR were classified either as intergenic (inter) or intragenic (intra) based on their position relative to known genes.

^e DMR were classified according to their CpG content using the promoter definitions proposed by Weber et al. (2005) as described in the Methods section.

^f Histon H3 lysin 4 methylation at DMR regions was classified as follows: Treg or Tconv Tri, increased H3K4 trimethylation in Treg or Tconv cells, respectively; Treg or Tconv Mono/Di, increased H3K4 mono-, or di-, but no trimethylation in Treg or Tconv cells, respectively; ND, H3K4 methylation present but not different between T cell subsets.

Supplemental Table S8
Sequence motifs associated with DMR showing enhancer activity

DMR class	TF Matrices	Number of Sequences ^a	Number of Matches ^b	Expected (genome) ^c	Std.dev.	Over representation (genome) ^d	Z-Score (genome) ^e
DMR showing no activity in the enhancer assay (n=12)	V\$STAT5.01	8	26	9.22	3.03	2.82	5.36
	V\$MYOD.01	7	10	2.59	1.61	3.86	4.29
	V\$NFKAPPAB65.01	9	15	5.03	2.24	2.98	4.22
	V\$HMX2.02	9	26	11.64	3.41	2.23	4.06
	V\$MZF1.02	8	20	8.26	2.87	2.42	3.91
	V\$STAT3.02	8	14	5.06	2.25	2.77	3.75
	V\$HEN1.02	3	7	1.72	1.31	4.08	3.65
	V\$GKLF.01	11	20	9.15	3.02	2.19	3.42
	V\$E47.01	8	8	2.34	1.53	3.41	3.37
	V\$ZID.01	5	8	2.38	1.54	3.36	3.31
	V\$MARE.02	4	8	2.43	1.56	3.29	3.25
	V\$NGN_NEUROD.01	7	11	4.24	2.06	2.59	3.04
	V\$NFKAPPAB.01	5	9	3.15	1.77	2.86	3.02
	V\$CREB2CJUN.01	2	4	0.28	0.53	14.06	6.03
DMR showing enhancer activity (n=12)	V\$ATF.01	7	10	1.85	1.36	5.4	5.62
	V\$ELK1.02	9	11	2.35	1.53	4.68	5.32
	V\$CTCF.03	5	12	2.92	1.71	4.11	5.02
	V\$HAND2_E12.01	4	11	2.71	1.65	4.06	4.73
	V\$ATF6.02	7	9	1.99	1.41	4.53	4.62
	V\$CTCF.04	5	10	2.39	1.55	4.18	4.59
	V\$CREB1.01	5	7	1.31	1.15	5.32	4.52
	V\$CREB.02	5	8	1.91	1.38	4.19	4.05
	V\$GABPB1.01	7	10	2.86	1.69	3.5	3.93
	V\$CETS1P54.01	9	13	4.42	2.1	2.94	3.85
	V\$KLF6.01	7	10	3	1.73	3.34	3.76
	V\$MZF1.03	6	15	5.79	2.41	2.59	3.62
	V\$STAT5.01	7	20	8.9	2.98	2.25	3.56
	V\$MZF1.01	6	15	5.98	2.45	2.51	3.48
	V\$E4F.01	7	13	4.89	2.21	2.66	3.44
	V\$HIF1.01	6	7	1.87	1.37	3.75	3.39
	V\$ETS2.01	11	19	8.71	2.95	2.18	3.32
	V\$RB_E2F1_DP1.01	4	8	2.42	1.55	3.31	3.27
	V\$USF.04	6	11	3.99	2	2.76	3.26
	V\$GKLF.01	11	19	8.83	2.97	2.15	3.25
	V\$E2F.02	10	11	4.07	2.02	2.7	3.18
	V\$ATF.02	9	11	4.11	2.03	2.68	3.16
	V\$OLF1.02	7	10	3.64	1.91	2.75	3.07
	V\$ROAZ.01	5	9	3.11	1.76	2.89	3.06

^a Number of input sequences with at least one match.

^b Number of matches in all sequences.

^c Expected match numbers in an equally sized sample of the genomic background and the standard deviation.

^d Overrepresentation against genomic background: Fold factor of match numbers in regions compared to an equally sized sample of the background (i.e. found versus expected).

^e Z-score of overrepresentation against genomic background: The distance from the population mean in units of the population standard deviation. Shown are only highly significant motifs (Z score >3; a Z-score above 2 can be considered statistically significant).