*Supplementary Material*

# Molecular markers distinguishing T cell subtypes with TSDR strand-bias methylation

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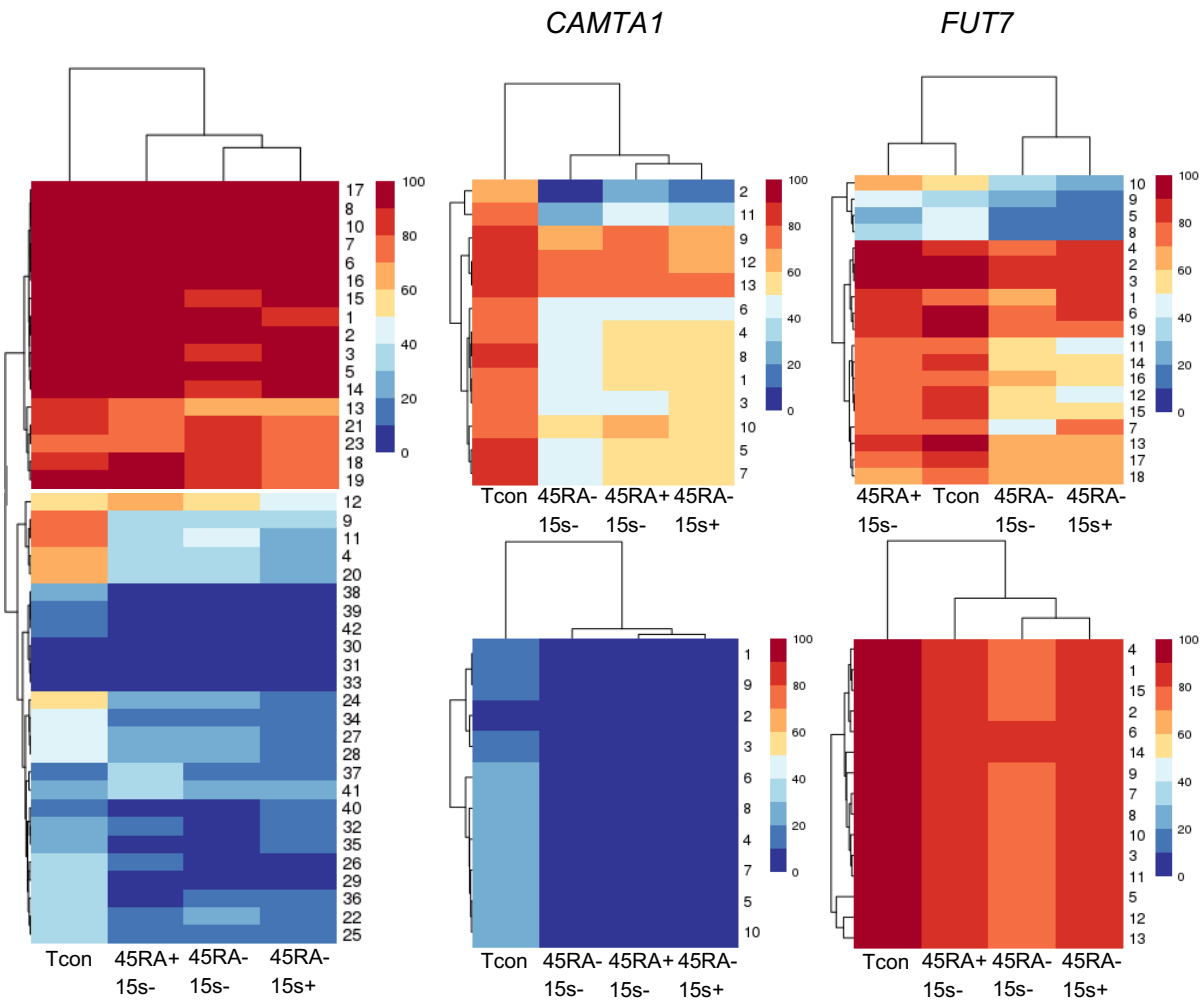
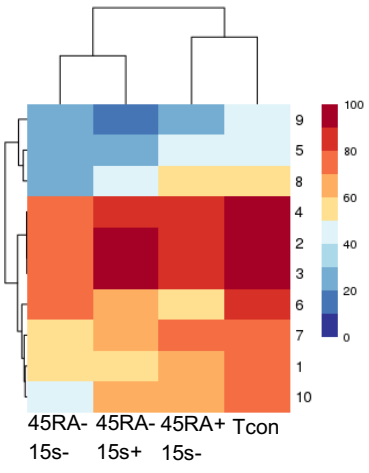
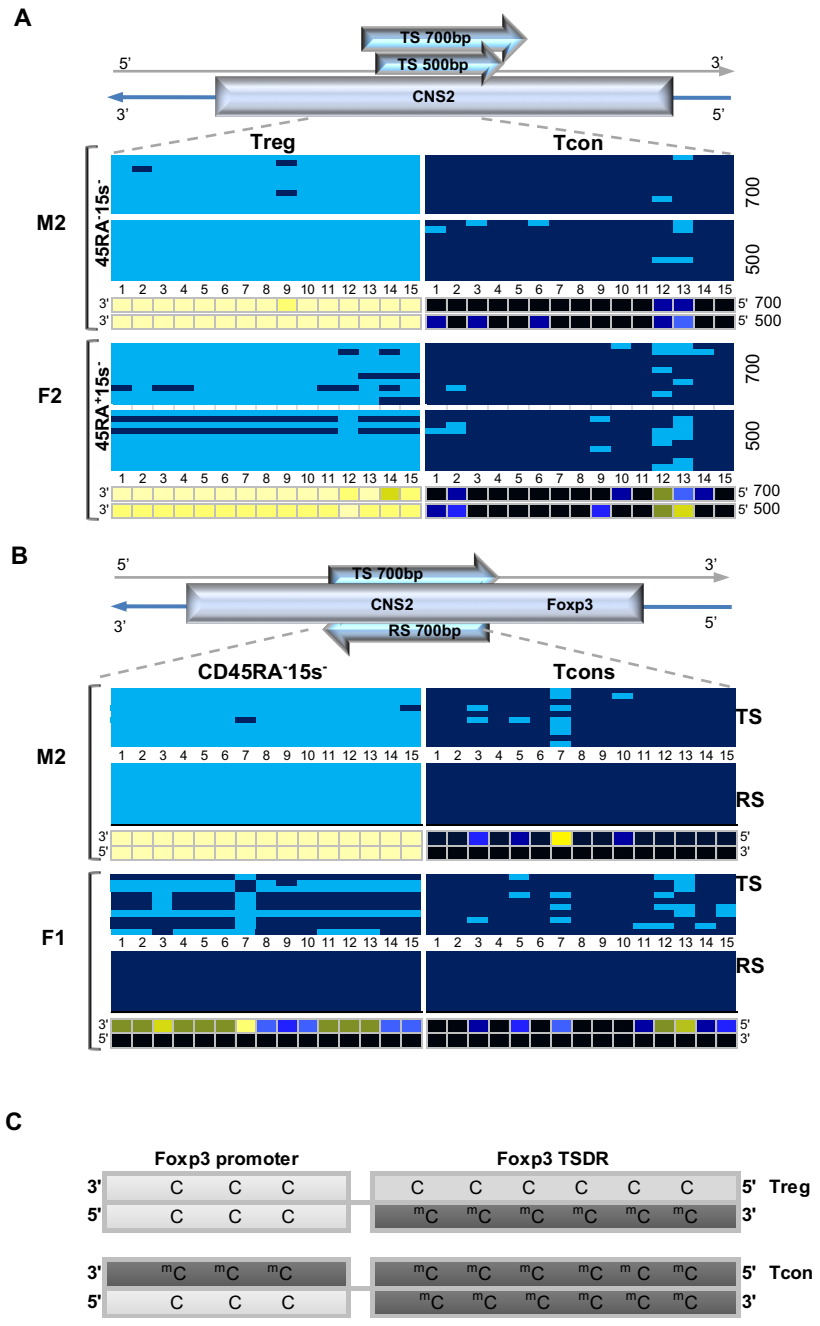
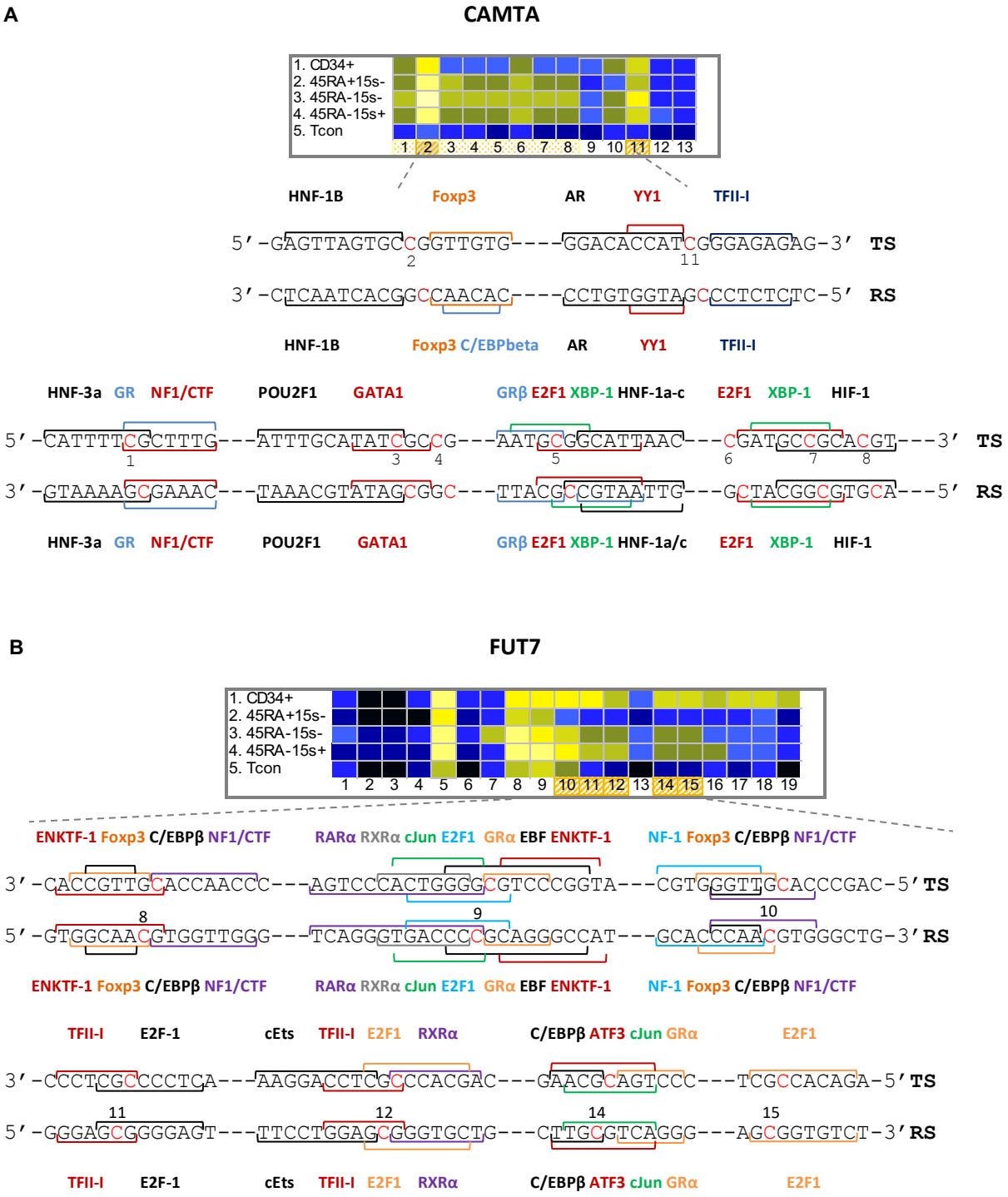


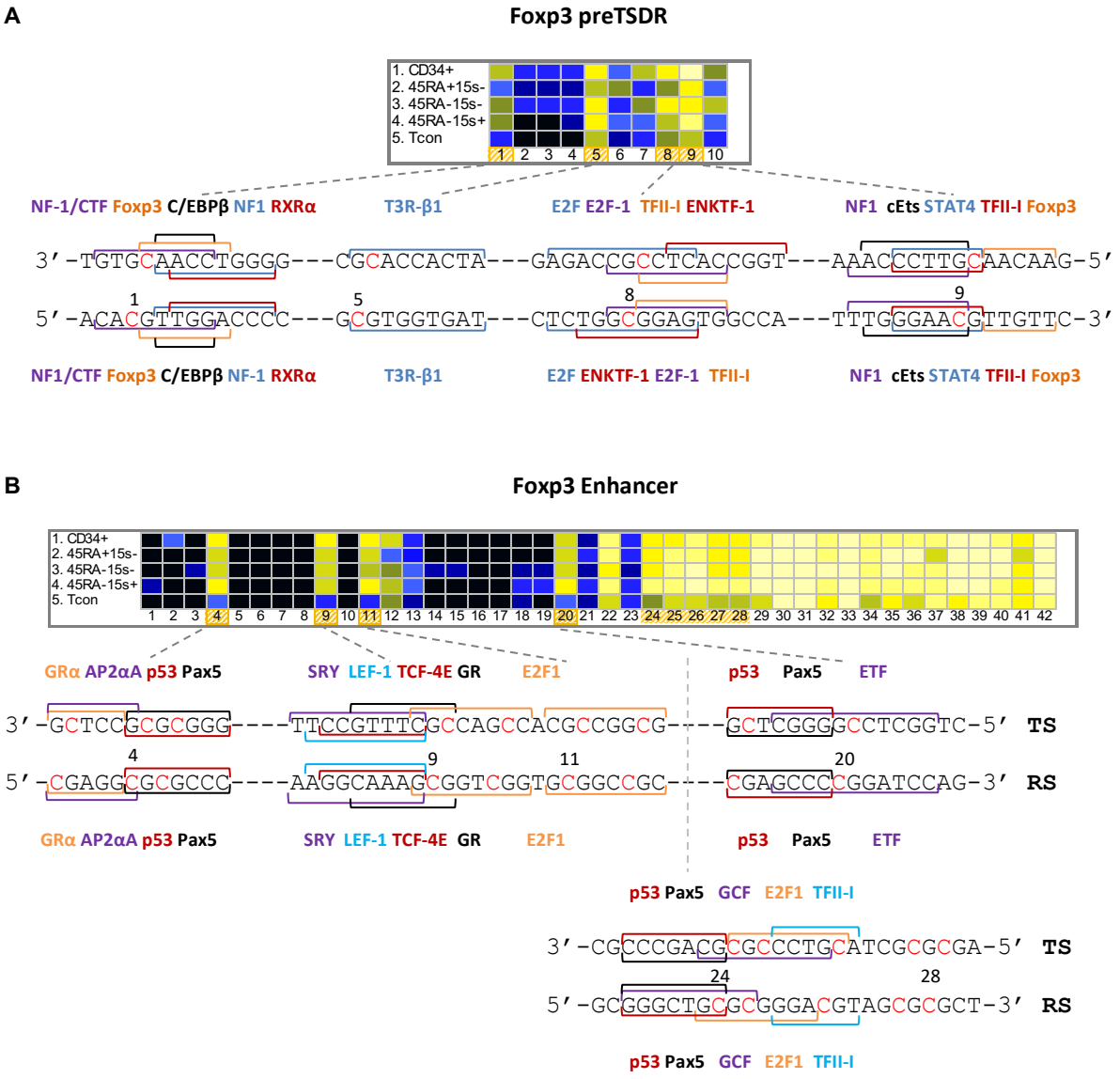

**Supplementary Figure 1.** Hierarchical clustering of Tcon and Treg ($45RA^{+}15s^{-}$, $45RA^{-}CD15s^{-}$ and $45RA^{-}15s^{+}$) subsets based on average methylation for each genomic element. The heatmap shows clustering based on cell types and CpG positions.



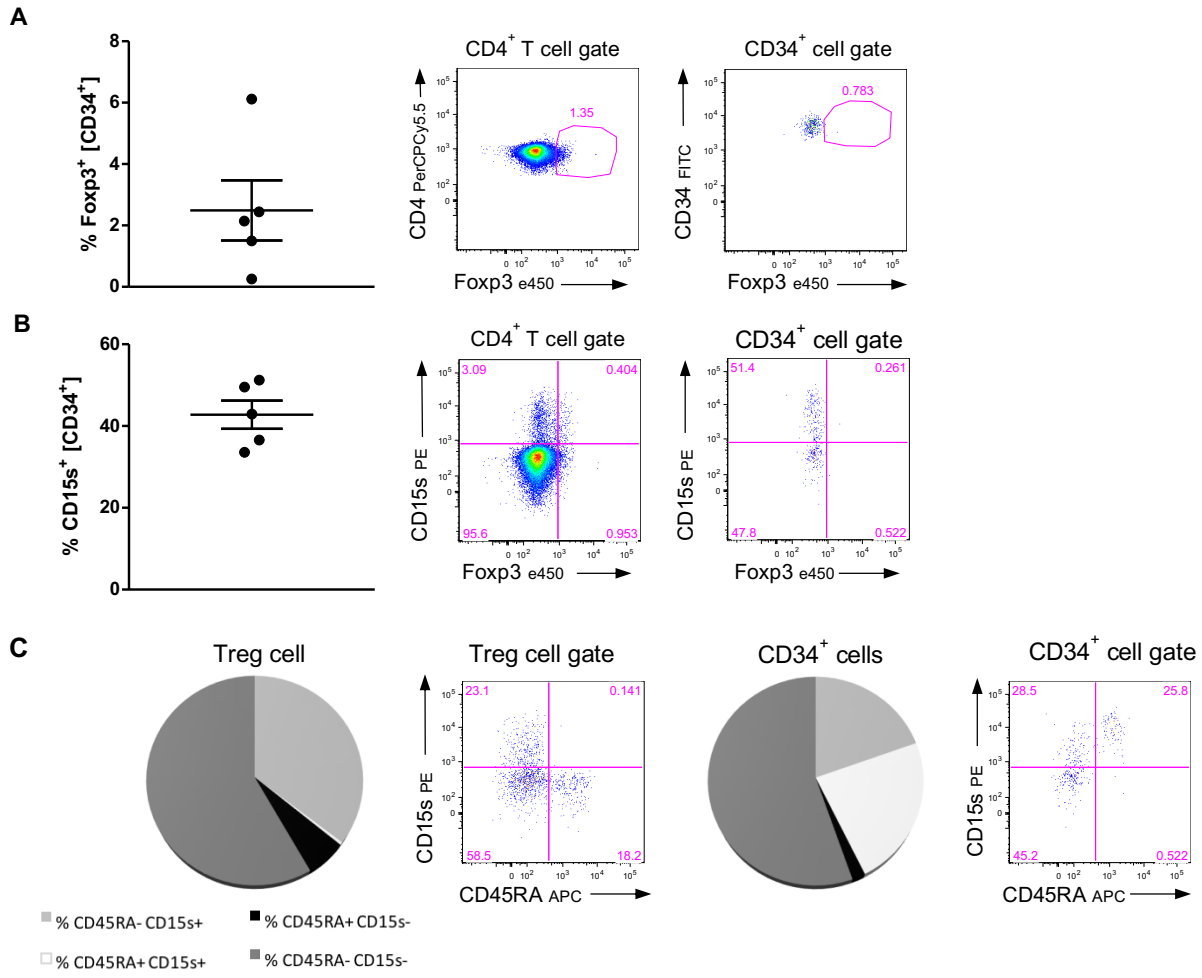

**Supplementary Figure 2.** Strand-specific methylation bias within the *FOXP3* TSDR region. (A). Methylation patterns of the top strand (TS) within TSDR of Treg and Tcon cells in 'conventional' (M2, top panel) and 'unconventional' (F2, lower panel) donors based on two amplicons, TS700bp and TS500bp. (B). Methylation patterns of the top strand (TS, upper panels) and reverse strand (RS, lower panels) within TSDR of $CD45RA^{-}CD15s^{-}$ and Tcon cells of donors M2 and F1. CpGs 1-15 are numbered relative to the 5'-3' direction of the coding (reverse) strand. Each horizontal line represents DNA from one cell with unmodified C in light blue and $^{m}C$ in dark blue. Methylation percentage for each CpG site was calculated based on the number of $^{m}Cs$ in a total of 10 and summarised in panels below. (C). Schematic presentation of 'unconventional' strand-bias methylation pattern in *FOXP3* promoter and TSDR of Treg and Tcon cells.



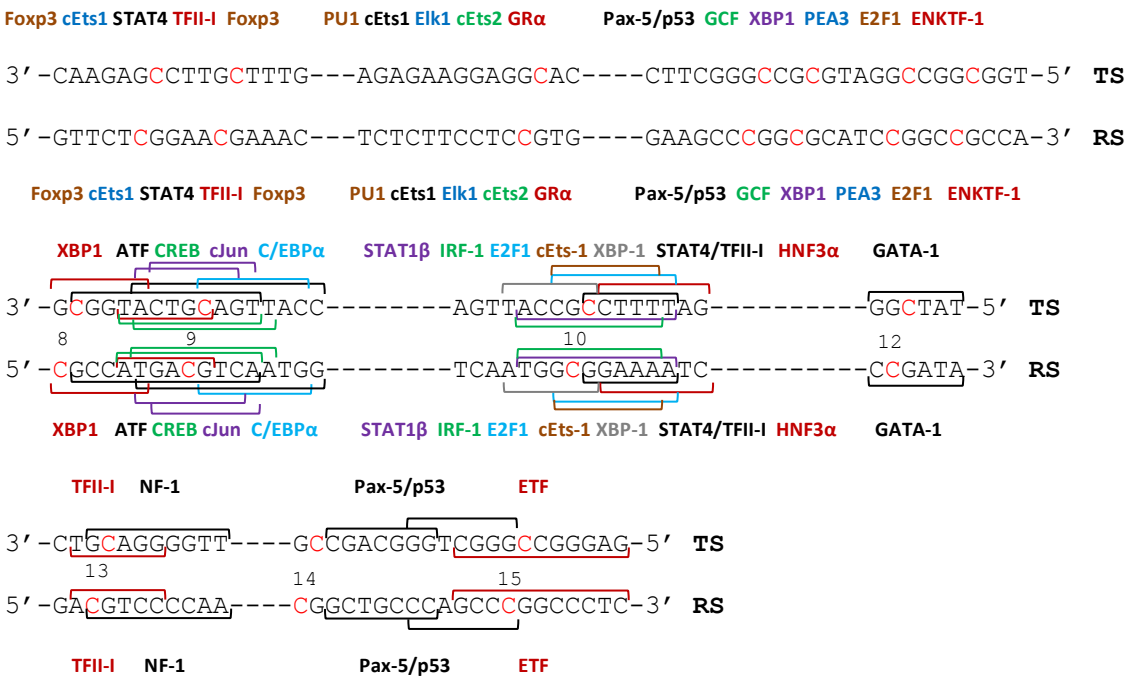

**Supplementary Figure 3.** Predicted TF binding sites containing or immediately adjacent to CpGs differentially methylated in *CAMTA1* and *FUT7* promoter of CD34+ cells and T lymphocyte subsets. Average methylation patterns for *CAMTA1* (A and Figure 2C) and *FUT7* (B and Figure 3C) are presented to indicate hypomethylated CpG sites. Coding strands of *CAMTA1* (TS) and *FUT7* (RS) are shown in the 5'-3' direction, Cs within 5'-CpG-3' are in red and numbered relative to the 5'-3' direction of the coding strand, TF recognition sequences are colour-coordinated with respective TFs. TS – top strand, RS – reverse strand




**Supplementary Figure 4.** Predicted TF binding sites containing or immediately adjacent to CpGs differentially methylated in *FOXP3* preTSDR and enhancer regions of CD34$^{+}$ cells and T lymphocyte subsets. Average methylation patterns for *FOXP3* preTSDR (A and Figure 5C) and enhancer (B and Figure 4C) are presented to indicate hypomethylated CpG sites. Coding (reverse) strands (RS) are shown in the 5'-3' direction, Cs within 5'-CpG-3' are in red and numbered relative to the 5'-3' direction of the coding strand, TF recognition sequences are colour-coordinated with respective TFs.




**Supplementary Figure 5**. Phenotypic analysis of CD34$^+$ cells by flow cytometry. Whole blood samples from donors M3, M6, F1, F2 and F4 were surface stained for CD34, CD3, CD4, CD25, CD127, CD45RA and CD15s. Following fixation and permeabilization, cells were incubated with anti-Foxp3 antibody. (A). The frequency of Foxp3$^+$ cells was determined within the CD34$^+$ population. Dot plots from a representative donor (M6) are shown, illustrating the expression of Foxp3 within CD4 T cells and CD34$^+$ cells. (B). The frequency of CD15s$^+$ cells was determined within the CD34$^+$ population. Dot plots from a representative donor (M6) are shown, illustrating the co-expression of CD15s and Foxp3 within CD4 T cells and CD34$^+$ cells. In the scatter plots, each symbol represents an individual (n=5), mean values are shown as horizontal lines and error bars represent the standard error of the mean. (C). The frequency of CD45RA/CD15s subsets was determined within Treg and CD34$^+$ cells. Pie charts show the average frequency of each subset within Treg and CD34$^+$ cells (n=5). Dot plots from a representative donor (M6) are shown, illustrating the distribution of CD45RA/CD15s subsets within Treg and CD34$^+$ cells.




**Supplementary Figure 6.** Differential binding of specific TFs to cytosines within CpG sites located on FOXP3 TSDR. While mapping to both strands, the predicted DNA binding sites of some TFs did not contain a cytosine (C) within their recognition sequences on one of the strands. Coding (reverse) strand (RS) is shown in the 5'-3' direction, Cs within 5'-CpG-3' are in red and numbered relative to 5'-3' direction of the coding strand, TF recognition sequences are colour-coordinated with respective TFs.

| Primer Name | Primer Sequence, 5'-3' | Amplicon size, bp | Strand |
|---|---|---|---|
| CAMTA Fw | GCG***CCATGG***AATAAGTAAAGAAAATTTAGTTGGTAATAAAATAAGGG | 470 | top (+) |
| CAMTA Rev | GCG***ATGCAT***ATAACAATCTCATCTAAATCAACCTATAACAAACACC | | top (+) |
| 1FUT7 Fw | GCG***CCATGG***TAATTTTAATATTTTGGGAGGTTGAAATTTGTGG | 500 | reverse (+) |
| 1FUT7 Rev | GCG***ATGCATT***CACCCTAAACCCCAACCAACCAACACC | | reverse (+) |
| 2FUT7 Fw | GCG***CCATGG***GTGGTTGGGTGAAGAGGTTGATGG | 454 | reverse (+) |
| 2FUT7 Rev | GCG***ATGCAT***CCCTTAATATCTTCCCACAAAAACC | | reverse (+) |
| Foxp3 Enhancer Fw | GCG***CCATGG***GGTTGTTGGTTTAGAAAGTGTT | 497 | reverse (+) |
| Foxp3 Enhancer Rev | GCG***ATGCAT***AAAATTTTACCTAATCCCCACATTTT | | reverse (+) |
| Foxp3 Promoter RS Fw | GCG***CCATGG***TTGTTTGTTTCGGGTTGGTTTTGTGATTTATTTTAG | 451 | reverse (+) |
| Foxp3 Promoter RS Rev | GCG***ATGCAT***ACAAAAAAAAAATCAACCTAACTT | | reverse (+) |
| Foxp3 Promoter TS Fw | GCG***CCATGG***TGTTATTTTTTTTAATGGGGTTTATATTTGGTAGGGG | 582 | top (-) |
| Foxp3 Promoter TS Rev | GCG***ATGCAT***CAAATTCTCTTTCTTTCCCCAAAAACC | | top (-) |
| Foxp3 preTSDR Fw | GCG***CCATGG***TTTTTTAGTATTTAGAAGGGGGAAGGGGAATTTGGG | 700 | reverse (+) |
| Foxp3 preTSDR Rev | GCG***ATGCAT***AAAACCCCTAAAATCTTCAAATAATATAACACCAAAAC | | reverse (+) |
| Foxp3 TSDR TS700 Fw | GCG***CCATGG***TATGTGGGATGGTTTGATTTAGTAAAGTATAG | 699 | top (-) |
| Foxp3 TSDR TS700 Rev | GCG***ATGCAT***TTTAAACACCAAAAACACTAACCTACACATAC | | top (-) |
| Foxp3 TSDR TS500 Fw | GCG***CCATGG***GGATATGGAGATGATTTGTTTGGGGGTAGAGG | 474 | top (-) |
| Foxp3 TSDR TS500 Rev | GCG***ATGCAT***CTAACACTCTCAAAACTTCAAACCTAAATCCTC | | top (-) |
| Foxp3 TSDR RS Fw | GCG***CCATGG***TTAGGGATATTGGTTTATATATATTGAGATTTTGG | 700 | reverse (+) |
| Foxp3 TSDR RS Rev | GCG***ATGCAT***CCCCAAACACATATAAAATAACCTAACTCAACAAAAC | | reverse (+) |

**Supplementary Table 1.** Bisulphite DNA strand-specific primers used for amplification of *CAMTA1*, *FUT7* and *FOXP3* gene regions for detection of methylation status. Coding strand is denoted as "+" (sense) and template strand is denoted as "-" (antisense). Nucleotides comprising restriction sites NcoI (forward primers, Fw) and NsiI (reverse primers, Rev) used for directional cloning are in bold italics

| Gene region | Position, 5'-3' | Primer position, 5'-3' | Amplicon size, bp | Strand |
|---|---|---|---|---|
| ***CAMTA1*,Ch1** | | | | |
| *CAMTA1* intronic region | 6,965,651-6,966,120 | Fw 6,965,651-6,965,689<br>Rev 6,966,120-6,966,083 | 470 | + |
| ***FUT7*, Ch9** | | | | |
| *FUT7* promoter 1 | 137,033,577-137,033,078 | Fw 137,033,577-137,033,551<br>Rev 137,033,078-137,033,105 | 500 | + |
| *FUT7* promoter 2 | 137,033,153-137,032,701 | Fw 137,033,153-137,033,131<br>Rev 137,032,701-137,033,725 | 454 | + |
| ***FOXP3*, ChX** | | | | |
| *FOXP3* Enhancer | 49,270,698-49,270,202 | Fw 49,270,698-49,270,676<br>Rev 49,270,202-49,270,228 | 497 | + |
| *FOXP3* Promoter RS | 49,265,128-49,264,678 | Fw 49,265,128-49,265,092<br>Rev 49,264,678-49,264,701 | 451 | + |
| *FOXP3* Promoter TS | 49,264,607-49,265,188 | Fw 49,264,607-49,264,643<br>Rev 49,265,188-49,265,156 | 582 | - |
| *FOXP3* preTSDR | 49,261,770-49,261,071 | Fw 49,261,770-49,261,734<br>Rev 49,261,071-49,261,109 | 700 | + |
| *FOXP3* TSDR TS700 | 49,260,531-49,261,229 | Fw 49,260,531-49,260,563<br>Rev 49,261,229-49,261,197 | 699 | - |
| *FOXP3* TSDR TS500 | 49,260,579-49,261,052 | Fw 49,260,579-49,260,611<br>Rev 49,261,052-49,261,019 | 474 | - |
| *FOXP3* TSDR RS | 49,261,221-49,260,522 | Fw 49,261,221-49,261,186<br>Rev 49,260,522-49,260,559 | 700 | + |

**Supplementary Table 2**. *CAMTA1*, *FUT7* and *FOXP3* gene regions used in the study of methylation status of the five cell populations. Fw – forward primer, Rev – reverse primer, TS-top strand, RS-reverse strand, "+" - sense (coding) strand, "-" – antisense (template) strand.

**CAMTA**

| Donor | | M1 | | | | | M2 | | | | | M3 | | | | | M4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 25 | 5 | 100 | 70 | N/A | 50 | 5 | 10 | 80 | N/A | 35 | 5 | 10 | 70 | 25 | 30 | 25 | 30 | 70 |
| 2 | 28 | N/A | 10 | 15 | 70 | 65 | N/A | 40 | 0 | 20 | 70 | N/A | 35 | 5 | 5 | 75 | 15 | 40 | 0 | 5 | 70 |
| 3 | 57 | N/A | 5 | 20 | 75 | 90 | N/A | 45 | 0 | 20 | 85 | N/A | 35 | 10 | 30 | 80 | 55 | 45 | 0 | 30 | 65 |
| 4 | 60 | N/A | 15 | 20 | 100 | 85 | N/A | 45 | 0 | 15 | 95 | N/A | 45 | 10 | 20 | 70 | 55 | 45 | 0 | 30 | 90 |
| 5 | 89 | N/A | 25 | 20 | 100 | 100 | N/A | 45 | 0 | 25 | 100 | N/A | 50 | 10 | 30 | 90 | 60 | 45 | 5 | 30 | 100 |
| 6 | 152 | N/A | 10 | 15 | 95 | 75 | N/A | 35 | 0 | 5 | 85 | N/A | 15 | 5 | 15 | 85 | 40 | 25 | 0 | 30 | 65 |
| 7 | 158 | N/A | 25 | 25 | 80 | 100 | N/A | 70 | 5 | 15 | 100 | N/A | 40 | 5 | 30 | 95 | 50 | 35 | 0 | 30 | 80 |
| 8 | 162 | N/A | 25 | 20 | 100 | 95 | N/A | 30 | 0 | 10 | 90 | N/A | 40 | 5 | 30 | 80 | 60 | 35 | 10 | 40 | 85 |
| 9 | 237 | N/A | 70 | 55 | 100 | 100 | N/A | 85 | 55 | 90 | 95 | N/A | 70 | 70 | 95 | 90 | 85 | 55 | 25 | 25 | 95 |
| 10 | 294 | N/A | 80 | 65 | 80 | 85 | N/A | 90 | 45 | 55 | 90 | N/A | 70 | 65 | 85 | 80 | 60 | 45 | 15 | 25 | 85 |
| 11 | 317 | N/A | 80 | 60 | 80 | 85 | N/A | 95 | 45 | 90 | 95 | N/A | 90 | 55 | 90 | 90 | 55 | 65 | 15 | 10 | 90 |
| 12 | 352 | N/A | 95 | 90 | 100 | 95 | N/A | 95 | 95 | 85 | 100 | N/A | 95 | 95 | 90 | 95 | 90 | 65 | 15 | 25 | 80 |
| 13 | 354 | N/A | 85 | 95 | 100 | 100 | N/A | 100 | 95 | 100 | 100 | N/A | 100 | 100 | 100 | 90 | 90 | 65 | 15 | 25 | 100 |
| Donor | | M5 | | | | | M6 | | | | | F1 | | | | | F2 | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 25 | 100 | 25 | 10 | 55 | 40 | 50 | 90 | 95 | 95 | 75 | 20 | 60 | 20 | 80 | 30 | 45 | 50 | 20 | 45 |
| 2 | 28 | 15 | 5 | 5 | 0 | 40 | 0 | 10 | 5 | 5 | 70 | 70 | 10 | 15 | 0 | 70 | 25 | 20 | 25 | 20 | 35 |
| 3 | 57 | 40 | 90 | 20 | 5 | 55 | 40 | 55 | 90 | 95 | 90 | 90 | 10 | 65 | 20 | 75 | 30 | 35 | 40 | 35 | 40 |
| 4 | 60 | 40 | 100 | 20 | 5 | 55 | 40 | 50 | 90 | 95 | 90 | 90 | 10 | 65 | 10 | 85 | 30 | 50 | 35 | 25 | 40 |
| 5 | 89 | 40 | 100 | 20 | 5 | 55 | 40 | 50 | 90 | 95 | 100 | 90 | 15 | 70 | 10 | 95 | 30 | 50 | 50 | 30 | 40 |
| 6 | 152 | 40 | 100 | 20 | 5 | 55 | 40 | 50 | 90 | 95 | 85 | 85 | 15 | 65 | 10 | 60 | 30 | 50 | 25 | 20 | 35 |
| 7 | 158 | 40 | 100 | 20 | 5 | 60 | 40 | 55 | 95 | 95 | 95 | 90 | 25 | 75 | 10 | 90 | 35 | 55 | 45 | 20 | 35 |
| 8 | 162 | 70 | 100 | 20 | 5 | 60 | 40 | 55 | 95 | 100 | 90 | 90 | 20 | 65 | 10 | 85 | 35 | 50 | 40 | 25 | 40 |
| 9 | 237 | 50 | 100 | 25 | 10 | 60 | 40 | 75 | 95 | 100 | 100 | 90 | 40 | 70 | 25 | 80 | 25 | 55 | 50 | 25 | 25 |
| 10 | 294 | 45 | 100 | 20 | 10 | 60 | 55 | 65 | 95 | 95 | 85 | 90 | 25 | 55 | 10 | 75 | 35 | 45 | 35 | 25 | 40 |
| 11 | 317 | 50 | 5 | 20 | 0 | 50 | 25 | 35 | 5 | 10 | 70 | 65 | 30 | 30 | 10 | 75 | 35 | 25 | 30 | 30 | 45 |
| 12 | 352 | 70 | 100 | 55 | 5 | 55 | 75 | 80 | 90 | 100 | 90 | 85 | 35 | 75 | 30 | 100 | 35 | 60 | 30 | 45 | 50 |
| 13 | 354 | 70 | 100 | 55 | 10 | 55 | 75 | 80 | 90 | 100 | 80 | 75 | 30 | 75 | 30 | 85 | 40 | 65 | 40 | 40 | 50 |
| Donor | | F3 | | | | | F4 | | | | | F5 | | | | | mean all donors | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 80 | 85 | 40 | 85 | 100 | 65 | 55 | 80 | 95 | 90 | 100 | 95 | 100 | 100 | 80 | 55 | 53.6 | 44.1 | 52.3 | 75.9 |
| 2 | 28 | 60 | 45 | 25 | 40 | 90 | 5 | 0 | 10 | 0 | 70 | 5 | 10 | 5 | 0 | 80 | 24.4 | 20.5 | 10 | 15 | 66.8 |
| 3 | 57 | 75 | 80 | 70 | 85 | 95 | 65 | 55 | 85 | 95 | 95 | 95 | 90 | 100 | 100 | 75 | 61.3 | 49.5 | 45.5 | 53.6 | 76.8 |
| 4 | 60 | 80 | 85 | 65 | 85 | 90 | 65 | 55 | 85 | 95 | 90 | 95 | 90 | 100 | 95 | 85 | 61.9 | 53.6 | 44.5 | 52.3 | 79.5 |
| 5 | 89 | 80 | 85 | 65 | 85 | 100 | 65 | 55 | 85 | 95 | 100 | 95 | 95 | 100 | 100 | 90 | 62.5 | 55.9 | 46.8 | 55 | 88.2 |
| 6 | 152 | 80 | 75 | 65 | 85 | 90 | 70 | 55 | 85 | 95 | 95 | 95 | 90 | 100 | 95 | 65 | 60 | 47.3 | 42.7 | 50 | 72.3 |
| 7 | 158 | 75 | 85 | 75 | 85 | 100 | 70 | 55 | 90 | 95 | 100 | 95 | 95 | 100 | 100 | 90 | 61.9 | 58.2 | 48.6 | 51.4 | 85.9 |
| 8 | 162 | 75 | 85 | 70 | 85 | 100 | 70 | 55 | 80 | 95 | 95 | 90 | 95 | 100 | 100 | 80 | 66.3 | 53.6 | 45.9 | 54.5 | 81.8 |
| 9 | 237 | 70 | 80 | 80 | 85 | 95 | 70 | 55 | 95 | 95 | 100 | 100 | 95 | 100 | 100 | 85 | 66.3 | 70.9 | 65.5 | 68.2 | 84.1 |
| 10 | 294 | 35 | 45 | 25 | 50 | 90 | 65 | 55 | 85 | 100 | 80 | 100 | 90 | 95 | 95 | 90 | 60.6 | 64.5 | 54.5 | 57.3 | 78.2 |
| 11 | 317 | 45 | 50 | 20 | 40 | 85 | 5 | 5 | 15 | 0 | 95 | 10 | 10 | 10 | 10 | 70 | 36.3 | 44.5 | 27.7 | 33.6 | 77.3 |
| 12 | 352 | 75 | 85 | 100 | 85 | 100 | 80 | 55 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 95 | 76.3 | 78.2 | 76.8 | 69.5 | 87.3 |
| 13 | 354 | 75 | 85 | 95 | 85 | 100 | 80 | 55 | 100 | 100 | 95 | 100 | 95 | 100 | 100 | 95 | 75.6 | 78.2 | 78.2 | 71.8 | 86.4 |
| mean CpGs 1-8 | | | | | | | | | | | | | | | | | **56.7** | **49** | **41** | **48** | **78.4** |
| mean all CpGs | | | | | | | | | | | | | | | | | **59.1** | **56** | **48.5** | **52.6** | **80** |

| Donor | | mean M1-4, F2 | | | | | mean M5,6, F1,3-5 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 27.5 | 37 | 18 | 34 | 67 | 64.2 | 67.5 | 65.8 | 67.5 | 83.3 |
| 2 | 28 | 20 | 29 | 9 | 24 | 63 | 25.8 | 13.3 | 10.8 | 7.5 | 70 |
| 3 | 57 | 42.5 | 33 | 14 | 38 | 72 | 67.5 | 63.3 | 71.7 | 66.7 | 80.8 |
| 4 | 60 | 42.5 | 40 | 13 | 38 | 76 | 68.3 | 65 | 70.8 | 64.2 | 82.5 |
| 5 | 89 | 45 | 43 | 17 | 43 | 86 | 68.3 | 66.7 | 71.7 | 65 | 90 |
| 6 | 152 | 35 | 27 | 9 | 33 | 69 | 68.3 | 64.2 | 70.8 | 64.2 | 75 |
| 7 | 158 | 42.5 | 45 | 16 | 35 | 82 | 68.3 | 69.2 | 75.8 | 65 | 89.2 |
| 8 | 162 | 47.5 | 36 | 15 | 41 | 78 | 72.5 | 68.3 | 71.7 | 65.8 | 85 |
| 9 | 237 | 55 | 67 | 51 | 67 | 81 | 70 | 74.2 | 77.5 | 69.2 | 86.7 |
| 10 | 294 | 47.5 | 66 | 45 | 54 | 76 | 65 | 63.3 | 62.5 | 60 | 80 |
| 11 | 317 | 45 | 71 | 41 | 60 | 81 | 33.3 | 22.5 | 16.7 | 11.7 | 74.2 |
| 12 | 352 | 62.5 | 82 | 65 | 69 | 84 | 80.8 | 75 | 86.7 | 70 | 90 |
| 13 | 354 | 65 | 83 | 69 | 73 | 88 | 79.2 | 74.2 | 85.8 | 70.8 | 85 |
| mean CpGs 1-8 | | **37.8** | **36.2** | **13.9** | **35.8** | **74.1** | **62.9** | **59.7** | **63.6** | **58.2** | **82** |
| mean all CpGs | | **44.4** | **50.7** | **29.4** | **46.8** | **77.2** | **64** | **60.5** | **64.5** | **57.5** | **82.4** |

**FUT7**

| Donor | | M1 | | | | | M2 | | | | | M3 | | | | | M4 | | | | | M5 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 90 | 35 | 45 | 75 | N/A | 65 | 55 | 90 | 70 | N/A | 75 | 75 | 60 | 45 | 70 | 55 | N/A | 90 | 80 | 35 | 100 | 90 | 90 | 85 |
| 2 | 65 | N/A | 100 | 80 | 55 | 90 | N/A | 85 | 90 | 90 | 95 | N/A | 75 | 100 | 80 | 80 | 90 | 85 | N/A | 100 | 100 | 80 | 100 | 100 | 90 | 100 |
| 3 | 95 | N/A | 100 | 95 | 70 | 80 | N/A | 95 | 85 | 100 | 90 | N/A | 90 | 95 | 70 | 75 | 100 | 85 | N/A | 100 | 100 | 85 | 95 | 100 | 90 | 100 |
| 4 | 167 | N/A | 100 | 85 | 45 | 65 | N/A | 75 | 70 | 90 | 95 | N/A | 95 | 90 | 70 | 80 | 70 | 85 | N/A | 100 | 90 | 55 | 100 | 100 | 90 | 95 |
| 5 | 250 | N/A | 75 | 45 | 10 | 45 | N/A | 50 | 10 | 0 | 50 | N/A | 60 | 40 | 25 | 50 | 30 | 20 | N/A | 20 | 70 | 5 | 5 | 0 | 0 | 65 |
| 6 | 266 | N/A | 90 | 80 | 20 | 90 | N/A | 70 | 80 | 95 | 95 | N/A | 90 | 85 | 70 | 90 | 80 | 60 | N/A | 100 | 90 | 55 | 95 | 80 | 90 | 95 |
| 7 | 288 | N/A | 75 | 40 | 15 | 60 | N/A | 70 | 45 | 75 | 70 | N/A | 70 | 70 | 65 | 60 | 75 | 50 | N/A | 80 | 85 | 55 | 85 | 70 | 90 | 90 |
| 8 | 306 | N/A | 90 | 30 | 10 | 55 | N/A | 70 | 15 | 10 | 70 | N/A | 75 | 40 | 25 | 50 | 25 | 15 | N/A | 20 | 80 | 15 | 5 | 0 | 20 | 50 |
| 9 | 387 | N/A | 65 | 50 | 20 | 30 | N/A | 60 | 20 | 10 | 60 | N/A | 50 | 15 | 5 | 45 | 0 | 15 | 35 | 10 | 50 | 0 | 10 | 5 | 30 | 20 |
| 10 | 419 | N/A | 70 | 40 | 10 | 30 | N/A | 75 | 10 | 0 | 70 | N/A | 60 | 10 | 10 | 55 | 0 | 20 | 25 | 10 | 60 | 10 | 90 | 100 | 70 | 55 |
| 11 | 480 | N/A | 100 | 95 | 55 | 80 | N/A | 95 | 75 | 80 | 90 | N/A | 100 | 50 | 35 | 100 | 0 | 30 | 25 | 30 | 85 | 45 | 90 | 90 | 70 | 30 |
| 12 | 524 | N/A | 100 | 85 | 60 | 80 | N/A | 100 | 85 | 90 | 95 | N/A | 90 | 75 | 20 | 95 | 65 | 25 | 70 | 30 | 95 | 70 | 20 | 5 | 5 | 10 |
| 13 | 543 | N/A | 100 | 95 | 45 | 85 | N/A | 100 | 80 | 85 | 90 | N/A | 95 | 40 | 20 | 100 | 60 | 25 | 65 | 35 | 100 | 80 | 100 | 20 | 95 | 95 |
| 14 | 549 | N/A | 95 | 90 | 35 | 80 | N/A | 100 | 90 | 80 | 95 | N/A | 100 | 70 | 30 | 100 | 45 | 25 | 35 | 45 | 95 | 45 | 95 | 20 | 55 | 40 |
| 15 | 642 | N/A | 100 | 90 | 60 | 95 | N/A | 100 | 80 | 100 | 100 | N/A | 100 | 75 | 25 | 100 | 45 | 20 | 60 | 25 | 85 | 50 | 35 | 15 | 10 | 10 |
| 16 | 667 | N/A | 100 | 80 | 60 | 80 | N/A | 100 | 60 | 95 | 90 | N/A | 100 | 55 | 30 | 75 | 45 | 15 | 50 | 25 | 90 | 45 | 95 | 100 | 60 | 50 |
| 17 | 707 | N/A | 95 | 100 | 80 | 85 | N/A | 100 | 80 | 95 | 95 | N/A | 100 | 60 | 30 | 70 | 25 | 10 | 50 | 55 | 95 | 40 | 95 | 100 | 90 | 70 |
| 18 | 729 | N/A | 85 | 95 | 85 | 85 | N/A | 65 | 75 | 85 | 65 | N/A | 80 | 55 | 10 | 95 | 25 | 10 | 45 | 45 | 80 | 35 | 100 | 100 | 100 | 100 |
| 19 | 731 | N/A | 100 | 100 | 100 | 90 | N/A | 100 | 90 | 100 | 100 | N/A | 75 | 35 | 10 | 95 | 50 | 10 | 60 | 60 | 95 | 45 | 100 | 100 | 100 | 100 |

| Donor | | M6 | | | | | F1 | | | | | F2 | | | | | F3 | | | | | mean all donors | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 80 | 95 | 100 | 100 | 95 | 100 | 95 | 100 | 85 | 100 | 95 | 73.8 | 83.6 | 67.1 | 81.4 | 78.8 |
| 2 | 65 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 95 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 91.3 | 92.1 | 85 | 87.9 | 95 |
| 3 | 95 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 85 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 93.8 | 95 | 85.8 | 90 | 91.3 |
| 4 | 167 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 90 | 90 | 100 | 100 | 95 | 100 | 100 | 100 | 85 | 100 | 95 | 78.8 | 93.6 | 80 | 84.3 | 88.8 |
| 5 | 250 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 0 | N/A | 10 | 0 | 0 | 15 | 25 | 5 | 20 | 0 | 15 | 10 | 55 | 13.8 | 30 | 17.9 | 12.9 | 43.8 |
| 6 | 266 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 90 | 90 | 100 | 100 | 100 | 100 | 95 | 100 | 95 | 100 | 100 | 80 | 86.4 | 78.6 | 82.1 | 93.8 |
| 7 | 288 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 30 | N/A | 70 | 90 | 100 | 10 | 80 | 90 | 75 | 100 | 80 | 90 | 80 | 73.8 | 78.6 | 49.3 | 70.7 | 75.6 |
| 8 | 306 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 10 | N/A | 15 | 5 | 10 | 15 | 20 | 10 | 65 | 0 | 15 | 10 | 60 | 27.5 | 37.9 | 17.9 | 16.4 | 48.8 |
| 9 | 387 | 20 | 25 | 20 | 10 | 30 | 5 | 90 | 0 | 0 | 30 | 50 | 35 | 45 | 55 | 60 | 55 | 60 | 25 | 5 | 10 | 21.7 | 45.6 | 23.9 | 16.1 | 37.2 |
| 10 | 419 | 30 | 35 | 45 | 15 | 70 | 5 | 100 | 0 | 45 | 55 | 55 | 25 | 70 | 70 | 65 | 75 | 90 | 45 | 30 | 80 | 29.2 | 62.8 | 38.3 | 28.9 | 60 |
| 11 | 480 | 20 | 40 | 60 | 20 | 95 | 5 | 100 | 0 | 0 | 65 | 45 | 55 | 80 | 60 | 60 | 45 | 75 | 45 | 25 | 95 | 26.7 | 76.1 | 57.8 | 41.7 | 77.8 |
| 12 | 524 | 10 | 45 | 55 | 10 | 85 | 0 | 100 | 25 | 90 | 90 | 60 | 85 | 90 | 80 | 85 | 65 | 85 | 45 | 55 | 90 | 45 | 72.2 | 59.4 | 48.9 | 80.6 |
| 13 | 543 | 60 | 60 | 60 | 35 | 100 | 10 | 100 | 25 | 100 | 85 | 80 | 85 | 90 | 95 | 85 | 90 | 100 | 80 | 70 | 95 | 63.3 | 85 | 61.7 | 64.4 | 92.8 |
| 14 | 549 | 15 | 45 | 60 | 20 | 100 | 0 | 100 | 30 | 100 | 80 | 70 | 85 | 90 | 65 | 85 | 65 | 65 | 40 | 55 | 100 | 40 | 78.9 | 58.3 | 53.9 | 86.1 |
| 15 | 642 | 25 | 40 | 50 | 25 | 90 | 0 | 100 | 30 | 100 | 85 | 60 | 90 | 80 | 85 | 80 | 60 | 80 | 50 | 35 | 90 | 40 | 73.9 | 58.9 | 51.7 | 81.7 |
| 16 | 667 | 35 | 30 | 75 | 20 | 100 | 0 | 100 | 20 | 45 | 75 | 55 | 60 | 80 | 75 | 70 | 65 | 85 | 60 | 80 | 90 | 40.8 | 76.1 | 64.4 | 54.4 | 80 |
| 17 | 707 | 35 | 35 | 70 | 25 | 95 | 0 | 100 | 5 | 100 | 90 | 60 | 80 | 60 | 60 | 70 | 65 | 80 | 75 | 95 | 95 | 37.5 | 77.2 | 66.7 | 70 | 85 |
| 18 | 729 | 55 | 55 | 65 | 40 | 90 | 0 | 100 | 5 | 100 | 80 | 40 | 50 | 75 | 50 | 25 | 50 | 80 | 90 | 40 | 85 | 34.2 | 69.4 | 67.2 | 61.7 | 78.3 |
| 19 | 731 | 50 | 60 | 70 | 40 | 90 | 0 | 100 | 20 | 100 | 85 | 65 | 80 | 95 | 90 | 75 | 85 | 100 | 100 | 95 | 90 | 49.2 | 80.6 | 74.4 | 77.2 | 91.1 |
| | | | | | | | | | | | | | | | | | | | mean CpGs 10-12,14,15 | | | **38.2** | **72.8** | **54.5** | **45** | **77.2** |
| | | | | | | | | | | | | | | | | | | | mean CpGs 8-19 | | | **37.9** | **69.6** | **54.1** | **48.8** | **75** |

**Foxp3 Enhancer**

| Donor | | M1 | | | | M2 | | | | M3 | | | | M4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #2 | #3 | #4 | #5 | #2 | #3 | #4 | #5 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 100 | 95 | 75 | 95 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 95 | 85 | 85 | 85 |
| 2 | 8 | 100 | 80 | 80 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0 | 100 | 100 | 100 | 95 |
| 3 | 14 | 65 | 65 | 70 | 85 | 90 | 80 | 100 | 80 | 95 | 100 | 90 | 95 | 100 | 95 | 100 | 95 | 95 |
| 4 | 19 | 95 | 90 | 25 | 95 | 75 | 25 | 0 | 85 | 5 | 5 | 10 | 45 | 20 | 35 | 25 | 20 | 85 |
| 5 | 21 | 80 | 60 | 80 | 100 | 100 | 95 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 95 |
| 6 | 29 | 75 | 70 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 95 |
| 7 | 33 | 70 | 90 | 70 | 95 | 100 | 90 | 100 | 95 | 95 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 90 |
| 8 | 35 | 100 | 70 | 80 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 9 | 67 | 95 | 95 | 25 | 100 | 70 | 25 | 0 | 100 | 10 | 5 | 10 | 55 | 0 | 35 | 25 | 35 | 85 |
| 10 | 71 | 95 | 95 | 90 | 100 | 100 | 90 | 100 | 100 | 100 | 95 | 100 | 95 | 100 | 100 | 100 | 100 | 95 |
| 11 | 76 | 85 | 90 | 25 | 100 | 75 | 60 | 0 | 95 | 10 | 10 | 0 | 55 | 0 | 35 | 25 | 35 | 80 |
| 12 | 80 | 90 | 85 | 40 | 80 | 0 | 10 | 0 | 20 | 65 | 20 | 15 | 35 | 0 | 95 | 95 | 95 | 95 |
| 13 | 83 | 85 | 75 | 75 | 100 | 40 | 35 | 55 | 95 | 60 | 55 | 60 | 75 | 70 | 95 | 95 | 100 | 80 |
| 14 | 86 | 75 | 55 | 80 | 95 | 100 | 75 | 100 | 100 | 95 | 100 | 90 | 100 | 100 | 95 | 100 | 100 | 100 |
| 15 | 89 | 100 | 90 | 80 | 95 | 100 | 5 | 100 | 100 | 95 | 100 | 100 | 95 | 100 | 95 | 100 | 100 | 95 |
| 16 | 93 | 100 | 85 | 80 | 100 | 90 | 100 | 100 | 100 | 100 | 100 | 95 | 95 | 100 | 95 | 100 | 100 | 100 |
| 17 | 101 | 100 | 90 | 80 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 95 | 100 | 100 | 100 |
| 18 | 113 | 75 | 50 | 90 | 90 | 80 | 85 | 100 | 80 | 100 | 100 | 90 | 85 | 100 | 95 | 100 | 100 | 80 |
| 19 | 125 | 70 | 60 | 70 | 90 | 90 | 75 | 100 | 90 | 95 | 100 | 90 | 90 | 100 | 95 | 100 | 100 | 95 |
| 20 | 132 | 55 | 75 | 15 | 80 | 70 | 5 | 0 | 75 | 0 | 0 | 0 | 30 | 25 | 35 | 40 | 40 | 75 |
| 21 | 168 | 45 | 60 | 80 | 75 | 100 | 80 | 95 | 75 | 100 | 100 | 100 | 80 | 80 | 100 | 100 | 95 | 100 |
| 22 | 180 | 20 | 40 | 0 | 30 | 10 | 0 | 0 | 60 | 0 | 0 | 0 | 30 | 0 | 30 | 20 | 35 | 50 |
| 23 | 183 | 50 | 75 | 65 | 65 | 100 | 85 | 95 | 100 | 100 | 100 | 90 | 85 | 100 | 30 | 80 | 85 | 55 |
| 24 | 190 | 55 | 40 | 0 | 65 | 45 | 5 | 0 | 75 | 0 | 0 | 0 | 40 | 20 | 30 | 20 | 35 | 55 |
| 25 | 192 | 20 | 25 | 0 | 25 | 40 | 10 | 0 | 35 | 0 | 0 | 0 | 40 | 20 | 30 | 20 | 35 | 50 |
| 26 | 197 | 20 | 15 | 0 | 25 | 45 | 0 | 0 | 50 | 0 | 0 | 0 | 25 | 10 | 30 | 10 | 20 | 50 |
| 27 | 202 | 55 | 40 | 0 | 60 | 35 | 15 | 0 | 60 | 0 | 0 | 0 | 35 | 20 | 30 | 20 | 35 | 50 |
| 28 | 204 | 45 | 35 | 0 | 60 | 65 | 10 | 0 | 50 | 0 | 0 | 0 | 30 | 20 | 30 | 20 | 40 | 55 |
| 29 | 215 | 25 | 5 | 10 | 35 | 5 | 5 | 0 | 35 | 0 | 5 | 0 | 10 | 25 | 0 | 10 | 25 | 50 |
| 30 | 233 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 0 |
| 31 | 236 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 | 5 |
| 32 | 247 | 5 | 5 | 0 | 0 | 40 | 0 | 0 | 20 | 5 | 0 | 0 | 15 | 0 | 0 | 5 | 15 | 55 |
| 33 | 251 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| 34 | 258 | 45 | 35 | 0 | 60 | 65 | 5 | 0 | 50 | 0 | 5 | 10 | 35 | 0 | 5 | 10 | 20 | 40 |
| 35 | 266 | 15 | 0 | 0 | 15 | 10 | 0 | 0 | 20 | 0 | 5 | 10 | 25 | 0 | 10 | 10 | 15 | 25 |
| 36 | 274 | 0 | 35 | 5 | 30 | 20 | 0 | 0 | 40 | 0 | 0 | 0 | 25 | 0 | 10 | 15 | 15 | 25 |
| 37 | 284 | 15 | 0 | 0 | 15 | 5 | 0 | 0 | 20 | 0 | 0 | 10 | 0 | 0 | 50 | 60 | 50 | 30 |
| 38 | 286 | 20 | 5 | 0 | 30 | 0 | 10 | 5 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
| 39 | 296 | 5 | 0 | 0 | 15 | 5 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 20 |
| 40 | 298 | 20 | 0 | 0 | 5 | 5 | 0 | 0 | 25 | 0 | 0 | 0 | 10 | 0 | 5 | 15 | 15 | 30 |
| 41 | 306 | 20 | 0 | 0 | 20 | 0 | 5 | 0 | 20 | 0 | 0 | 0 | 10 | 0 | 65 | 70 | 35 | 45 |
| 42 | 309 | 20 | 5 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 5 | 0 | 5 | 5 | 0 | 15 |

**Foxp3 Enhancer**

| Donor | | F1 | | | | | F2 | | | | | mean all donors | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 95 | 90 | 85 | 90 | 95 | 90 | 90 | 95 | 80 | 80 | 95 | 96.3 | 93.3 | 88.3 | 91.7 |
| 2 | 8 | 100 | 100 | 95 | 90 | 95 | 100 | 100 | 95 | 80 | 80 | 66.8 | 100 | 95 | 91.7 | 95 |
| 3 | 14 | 100 | 100 | 95 | 90 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 90.9 | 90 | 90.9 | 90.9 |
| 4 | 19 | 35 | 20 | 30 | 55 | 55 | 25 | 0 | 35 | 40 | 35 | 26.8 | 38.3 | 35 | 25 | 66.7 |
| 5 | 21 | 100 | 100 | 95 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 96.7 | 91.7 | 95 | 98.3 |
| 6 | 29 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 95.9 | 95 | 96.7 | 98.3 |
| 7 | 33 | 100 | 100 | 95 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 94.1 | 95.9 | 93.3 | 95.9 |
| 8 | 35 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 96.7 | 98.3 |
| 9 | 67 | 35 | 20 | 25 | 55 | 55 | 35 | 5 | 35 | 60 | 80 | 23.3 | 39.1 | 35 | 30.9 | 79.1 |
| 10 | 71 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 99.1 | 96.7 | 98.3 | 98.3 |
| 11 | 76 | 45 | 20 | 25 | 55 | 60 | 35 | 5 | 35 | 60 | 80 | 26.8 | 38.3 | 40.9 | 29.1 | 78.3 |
| 12 | 80 | 5 | 15 | 0 | 5 | 0 | 100 | 100 | 95 | 95 | 100 | 35 | 60.9 | 50.9 | 41.7 | 55 |
| 13 | 83 | 70 | 60 | 65 | 5 | 85 | 100 | 100 | 90 | 95 | 100 | 80 | 73.3 | 69.1 | 65 | 89.1 |
| 14 | 86 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 94.1 | 87.4 | 95 | 99.1 |
| 15 | 89 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 95 | 80 | 80 | 100 | 98.3 | 81.7 | 93.3 | 94.1 |
| 16 | 93 | 100 | 95 | 100 | 100 | 95 | 100 | 100 | 95 | 100 | 100 | 100 | 96.7 | 96.7 | 95.9 | 98.3 |
| 17 | 101 | 100 | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 99.1 | 98.3 | 95.9 | 98.3 |
| 18 | 113 | 100 | 100 | 100 | 0 | 90 | 100 | 100 | 100 | 100 | 100 | 100 | 91.7 | 89.1 | 80 | 85.4 |
| 19 | 125 | 100 | 100 | 100 | 0 | 95 | 100 | 100 | 95 | 100 | 100 | 100 | 91.7 | 88.3 | 76.7 | 93.3 |
| 20 | 132 | 45 | 20 | 25 | 55 | 60 | 30 | 5 | 40 | 65 | 75 | 33.3 | 30.9 | 30.9 | 29.1 | 65.9 |
| 21 | 168 | 100 | 15 | 100 | 0 | 85 | 90 | 100 | 95 | 100 | 90 | 90 | 76.7 | 89.1 | 78.3 | 84.1 |
| 22 | 180 | 30 | 20 | 20 | 45 | 45 | 15 | 0 | 45 | 35 | 15 | 15 | 13.3 | 20.9 | 19.1 | 38.3 |
| 23 | 183 | 30 | 85 | 100 | 65 | 70 | 100 | 100 | 75 | 75 | 100 | 76.8 | 77.4 | 85.9 | 79.1 | 79.1 |
| 24 | 190 | 45 | 20 | 20 | 45 | 55 | 15 | 0 | 45 | 35 | 35 | 26.8 | 25 | 21.7 | 19.1 | 54.1 |
| 25 | 192 | 40 | 20 | 20 | 45 | 45 | 15 | 0 | 45 | 35 | 35 | 25 | 18.3 | 20 | 19.1 | 38.3 |
| 26 | 197 | 20 | 5 | 20 | 10 | 35 | 15 | 0 | 0 | 10 | 20 | 15 | 16.7 | 7.4 | 6.7 | 34.1 |
| 27 | 202 | 45 | 20 | 20 | 50 | 45 | 15 | 0 | 45 | 25 | 15 | 26.8 | 23.3 | 23.3 | 18.3 | 44.1 |
| 28 | 204 | 45 | 20 | 20 | 50 | 45 | 15 | 0 | 45 | 25 | 15 | 26.8 | 26.7 | 21.6 | 19.1 | 42.4 |
| 29 | 215 | 15 | 10 | 20 | 10 | 35 | 15 | 0 | 10 | 10 | 35 | 18.3 | 6.7 | 9.1 | 9.1 | 33.3 |
| 30 | 233 | 0 | 5 | 0 | 10 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0.7 | 7.4 | 2.4 | 5 |
| 31 | 236 | 0 | 5 | 0 | 10 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 2.4 | 5.9 |
| 32 | 247 | 30 | 15 | 0 | 45 | 35 | 10 | 0 | 45 | 45 | 30 | 13.3 | 10.9 | 9.1 | 17.4 | 26.3 |
| 33 | 251 | 0 | 5 | 0 | 5 | 15 | 0 | 5 | 0 | 0 | 0 | 0 | 4.1 | 0 | 0.7 | 3.3 |
| 34 | 258 | 40 | 5 | 0 | 45 | 45 | 10 | 0 | 35 | 45 | 40 | 16.8 | 20 | 15 | 20 | 45 |
| 35 | 266 | 30 | 20 | 0 | 45 | 35 | 10 | 0 | 40 | 45 | 30 | 13.3 | 9.1 | 9.1 | 19.1 | 25 |
| 36 | 274 | 30 | 20 | 0 | 45 | 30 | 10 | 0 | 50 | 45 | 40 | 13.3 | 8.3 | 16.7 | 18.3 | 31.7 |
| 37 | 284 | 15 | 80 | 0 | 5 | 20 | 40 | 50 | 25 | 15 | 35 | 18.3 | 33.3 | 14.1 | 13.3 | 20 |
| 38 | 286 | 15 | 10 | 0 | 5 | 25 | 10 | 0 | 20 | 10 | 35 | 8.3 | 5 | 5.9 | 3.3 | 21.7 |
| 39 | 296 | 15 | 10 | 0 | 5 | 20 | 10 | 0 | 20 | 10 | 35 | 8.3 | 3.3 | 4.1 | 2.4 | 19.1 |
| 40 | 298 | 45 | 20 | 0 | 45 | 30 | 0 | 0 | 40 | 35 | 10 | 15 | 8.3 | 9.1 | 15.9 | 18.3 |
| 41 | 306 | 45 | 90 | 0 | 45 | 30 | 30 | 50 | 55 | 55 | 45 | 25 | 37.6 | 21.7 | 22.4 | 28.3 |
| 42 | 309 | 15 | 0 | 0 | 5 | 15 | 10 | 0 | 10 | 10 | 50 | 8.3 | 4.1 | 3.3 | 2.4 | 16.7 |
| **mean CpGs 4,9,11,20** | | **all donors** | | | | | | | | | | **27.6** | **36.7** | **35.5** | **28.5** | **72.5** |
| **mean CpGs 4,9,11,20** | | **donor M4** | | | | | | | | | | **11.2** | **35** | **28.7** | **32.5** | **81.2** |

**Foxp3 preTSDR**

| Donor | | M2 | | | | | M4 | | | | | M5 | | | | | M6 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 90 | 75 | 90 | 85 | 45 | 100 | 10 | 0 | 75 | 85 | 65 | 60 | 45 | 55 | 15 | 35 | 85 | 60 | 75 |
| 2 | 30 | N/A | 100 | 80 | 100 | 100 | 95 | 100 | 80 | 75 | 100 | 100 | 95 | 85 | 90 | 90 | 100 | 100 | 100 | 100 | 100 |
| 3 | 37 | N/A | 100 | 80 | 100 | 100 | 95 | 100 | 90 | 75 | 100 | 100 | 95 | 85 | 90 | 90 | 100 | 100 | 100 | 100 | 95 |
| 4 | 87 | N/A | 100 | 80 | 95 | 100 | 95 | 100 | 80 | 95 | 95 | 95 | 95 | 80 | 90 | 90 | 100 | 100 | 100 | 100 | 95 |
| 5 | 123 | N/A | 40 | 15 | 10 | 10 | 30 | 100 | 15 | 0 | 70 | 20 | 50 | 65 | 70 | 70 | 15 | 20 | 0 | 65 | 85 |
| 6 | 187 | N/A | 65 | 75 | 80 | 100 | 70 | 0 | 70 | 70 | 75 | 80 | 90 | 75 | 85 | 80 | 85 | 45 | 95 | 30 | 75 |
| 7 | 243 | N/A | 95 | 75 | 90 | 85 | 30 | 100 | 10 | 0 | 65 | 85 | 45 | 60 | 45 | 55 | 15 | 30 | 90 | 70 | 85 |
| 8 | 334 | N/A | 50 | 15 | 10 | 10 | 30 | 100 | 10 | 0 | 80 | 25 | 75 | 80 | 80 | 75 | 15 | 15 | 5 | 65 | 85 |
| 9 | 357 | N/A | 10 | 10 | 0 | 10 | 5 | 0 | 0 | 0 | 60 | 0 | 50 | 60 | 45 | 50 | 0 | 15 | 0 | 0 | 60 |
| 10 | 524 | N/A | 55 | 10 | 25 | 45 | 90 | 100 | 85 | 80 | 100 | 30 | 90 | 85 | 90 | 95 | 100 | 75 | 20 | 95 | 90 |
| Donor | | F1 | | | | | F2 | | | | | F3 | | | | | F4 | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 10 | 70 | 25 | 0 | 75 | 10 | 75 | 30 | 100 | 90 | 55 | 25 | 50 | 45 | 70 | 75 | 80 | 65 | 75 | 60 |
| 2 | 30 | 10 | 100 | 45 | 100 | 100 | 25 | 90 | 55 | 100 | 100 | 100 | 25 | 55 | 80 | 100 | 95 | 90 | 90 | 90 | 100 |
| 3 | 37 | 20 | 100 | 45 | 100 | 100 | 25 | 90 | 65 | 100 | 100 | 100 | 20 | 50 | 80 | 95 | 95 | 90 | 95 | 90 | 100 |
| 4 | 87 | 15 | 100 | 60 | 95 | 100 | 15 | 85 | 55 | 90 | 95 | 90 | 30 | 55 | 80 | 90 | 100 | 85 | 90 | 85 | 100 |
| 5 | 123 | 0 | 95 | 10 | 0 | 10 | 0 | 35 | 25 | 10 | 20 | 60 | 25 | 30 | 45 | 85 | 15 | 20 | 15 | 30 | 70 |
| 6 | 187 | 5 | 30 | 50 | 100 | 100 | 15 | 65 | 60 | 70 | 95 | 50 | 40 | 60 | 35 | 90 | 100 | 70 | 90 | 80 | 80 |
| 7 | 243 | 10 | 90 | 35 | 95 | 75 | 15 | 90 | 45 | 100 | 85 | 60 | 40 | 50 | 50 | 95 | 80 | 90 | 75 | 75 | 80 |
| 8 | 334 | 10 | 100 | 20 | 95 | 20 | 0 | 45 | 30 | 25 | 30 | 80 | 50 | 40 | 50 | 100 | 35 | 40 | 10 | 55 | 75 |
| 9 | 357 | 0 | 25 | 95 | 5 | 20 | 0 | 30 | 15 | 0 | 35 | 15 | 40 | 25 | 15 | 90 | 20 | 10 | 5 | 20 | 55 |
| 10 | 524 | 10 | 100 | 50 | 100 | 45 | 10 | 60 | 30 | 20 | 65 | 95 | 35 | 60 | 75 | 100 | 60 | 40 | 45 | 40 | 80 |
| Donor | | F5 | | | | | mean all donors | | | | | 70.5 | 33 | 47.5 | 55.5 | 91.5 | mean all CpGs donor F3 | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | | | | | | | | | | |
| 1 | 1 | 70 | 40 | 95 | 55 | 100 | 45.6 | 64.4 | 55 | 52.2 | 76.1 | | | | | | | | | | |
| 2 | 30 | 95 | 75 | 100 | 80 | 100 | 77.5 | 86.1 | 76.7 | 90.6 | 98.9 | | | | | | | | | | |
| 3 | 37 | 95 | 75 | 95 | 80 | 100 | 78.8 | 85.6 | 78.3 | 90.6 | 97.8 | | | | | | | | | | |
| 4 | 87 | 85 | 75 | 90 | 80 | 95 | 74.4 | 85.6 | 76.7 | 90 | 95.6 | | | | | | | | | | |
| 5 | 123 | 30 | 45 | 35 | 20 | 15 | 21.3 | 47.8 | 23.3 | 27.8 | 48.3 | | | | | | | | | | |
| 6 | 187 | 80 | 70 | 60 | 70 | 95 | 60.6 | 52.8 | 70.6 | 68.9 | 87.8 | | | | | | | | | | |
| 7 | 243 | 80 | 75 | 100 | 70 | 90 | 46.9 | 72.8 | 60 | 66.1 | 79.4 | | | | | | | | | | |
| 8 | 334 | 35 | 45 | 55 | 25 | 30 | 28.8 | 57.8 | 29.4 | 45 | 56.1 | | | | | | | | | | |
| 9 | 357 | 30 | 40 | 15 | 20 | 20 | 8.8 | 24.4 | 25 | 11.7 | 44.4 | | | | | | | | | | |
| 10 | 524 | 50 | 50 | 55 | 30 | 45 | 55.6 | 67.2 | 48.9 | 61.7 | 73.9 | | | | | | | | | | |
| mean CpGs 5,8,9 all donors | | | | | | | 19.6 | 43.3 | 25.9 | 28.2 | 49.6 | | | | | | | | | | |
| mean CpGs 5,8,9 donor M6 | | | | | | | 10 | 16.7 | 1.7 | 43.3 | 76.7 | | | | | | | | | | |

## Foxp3 promoter

| Donor | | M1 | | | | | M2 | | | | | M3 | | | | | M4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 15 | 0 | 15 | 60 | N/A | 0 | 0 | 0 | 30 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 41 | N/A | 15 | 0 | 5 | 45 | N/A | 0 | 0 | 0 | 25 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 118 | N/A | 15 | 5 | 10 | 55 | N/A | 0 | 0 | 0 | 25 | N/A | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 5 | 0 |
| 4 | 130 | N/A | 15 | 0 | 10 | 75 | N/A | 5 | 0 | 0 | 65 | N/A | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 |
| 5 | 143 | N/A | 15 | 10 | 20 | 70 | N/A | 5 | 0 | 0 | 70 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 179 | N/A | 10 | 10 | 20 | 85 | N/A | 15 | 0 | 0 | 75 | N/A | 0 | 0 | 5 | 15 | 5 | 0 | 5 | 0 | 0 |
| 7 | 191 | N/A | 15 | 5 | 15 | 60 | N/A | 15 | 0 | 0 | 70 | N/A | 0 | 0 | 5 | 10 | 0 | 0 | 0 | 0 | 0 |
| 8 | 198 | N/A | 10 | 0 | 25 | 75 | N/A | 5 | 0 | 0 | 65 | N/A | 0 | 0 | 0 | 15 | 0 | 5 | 0 | 5 | 0 |
| 9 | 213 | N/A | 0 | 0 | 15 | 60 | N/A | 5 | 0 | 0 | 25 | N/A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 241 | N/A | 15 | 5 | 15 | 70 | N/A | 5 | 0 | 0 | 55 | N/A | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 |
| Donor | | M5 | | | | | M6 | | | | | F1 | | | | | F2 | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 5 |
| 2 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 |
| 3 | 118 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 5 | 0 | 5 | 0 | 0 |
| 4 | 130 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 5 | 0 | 0 | 0 | 0 |
| 5 | 143 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 5 | 0 | 0 | 0 | 5 |
| 6 | 179 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 5 | 0 | 0 | 0 | 0 |
| 7 | 191 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| 8 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 |
| 9 | 213 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 10 | 0 | 0 | 0 |
| 10 | 241 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 |
| Donor | | F3 | | | | | mean all donors | | | | | mean M1 and 2 | | | | | mean M3-6 and F1-5 | | | | |
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1.7 | 0 | 1.7 | 13.9 | N/A | 7.5 | 0 | 7.5 | 45 | 0 | 0 | 0 | 0 | 5 |
| 2 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 1.7 | 0 | 0.6 | 10 | N/A | 7.5 | 0 | 2.5 | 35 | 0 | 0 | 0 | 0 | 2.9 |
| 3 | 118 | 0 | 0 | 0 | 0 | 0 | 1.7 | 1.7 | 1.1 | 1.7 | 11.1 | N/A | 7.5 | 2.5 | 5 | 40 | 1.7 | 0 | 0.7 | 0.7 | 2.9 |
| 4 | 130 | 0 | 0 | 0 | 0 | 0 | 0.8 | 2.2 | 0 | 1.1 | 21.7 | N/A | 10 | 0 | 5 | 70 | 0.8 | 0 | 0 | 0 | 7.9 |
| 5 | 143 | 0 | 0 | 0 | 0 | 0 | 0.8 | 2.2 | 1.1 | 2.2 | 20.6 | N/A | 10 | 5 | 10 | 70 | 0.8 | 0 | 0 | 0 | 6.4 |
| 6 | 179 | 0 | 0 | 0 | 0 | 0 | 1.7 | 2.8 | 1.7 | 2.8 | 25 | N/A | 12.5 | 5 | 10 | 80 | 1.7 | 0 | 0.7 | 0.7 | 9.3 |
| 7 | 191 | 0 | 0 | 0 | 0 | 0 | 0 | 3.3 | 0.6 | 2.2 | 21.1 | N/A | 15 | 2.5 | 7.5 | 65 | 0 | 0 | 0 | 0.7 | 8.6 |
| 8 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | 2.2 | 0 | 3.3 | 21.7 | N/A | 7.5 | 0 | 12.5 | 70 | 0 | 0.7 | 0 | 0.7 | 7.9 |
| 9 | 213 | 0 | 0 | 0 | 0 | 0 | 0 | 1.7 | 0 | 1.7 | 14.4 | N/A | 2.5 | 0 | 7.5 | 42.5 | 0 | 1.4 | 0 | 0 | 6.4 |
| 10 | 241 | 0 | 0 | 0 | 0 | 0 | 0 | 2.2 | 0.6 | 1.7 | 20.6 | N/A | 10 | 2.5 | 7.5 | 62.5 | 0 | 0 | 0 | 0 | 8.6 |
| mean all CpGs | | | | | | | 0.5 | 2.2 | 0.5 | 1.9 | 18 | N/A | 9 | 1.7 | 7.5 | 58 | 0.5 | 0.2 | 0.1 | 0.3 | 6.6 |

| Foxp3 Promoter Top/Reverse Strands | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Donor | | M6 | | | F3 | | F4 | | |
| | subset | #2 | #5 | #5 | #2 | #5 | #2 | #5 | #5 |
| CpG | nt | TS | TS | RS | TS | TS | TS | TS | RS |
| 1 | 1 | 0 | 60 | 0 | 60 | 80 | 60 | 40 | 0 |
| 2 | 41 | 0 | 50 | 0 | 70 | 40 | 0 | 40 | 0 |
| 3 | 118 | 0 | 60 | 0 | 90 | 50 | 60 | 30 | 0 |
| 4 | 130 | 10 | 100 | 0 | 80 | 70 | 60 | 80 | 0 |
| 5 | 143 | 10 | 100 | 0 | 80 | 90 | 60 | 90 | 0 |
| 6 | 179 | 10 | 100 | 0 | 0 | 50 | 0 | 0 | 0 |
| 7 | 191 | 0 | 90 | 0 | 80 | 100 | 60 | 100 | 0 |
| 8 | 198 | 10 | 100 | 0 | 80 | 70 | 60 | 100 | 0 |
| 9 | 213 | 0 | 90 | 0 | 50 | 100 | 60 | 80 | 0 |
| 10 | 241 | 10 | 90 | 0 | 40 | 70 | 60 | 80 | 0 |
| mean all CpGs | | 5 | 84 | 0 | 63 | 72 | 48 | 64 | 0 |

**Foxp3 TSDR**

| Donor | | M1 | | | | | M2 | | | | | M3 | | | | | M4 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 25 | 0 | 10 | 95 | N/A | 75 | 0 | 15 | 80 | N/A | 85 | 100 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 2 | 6 | N/A | 25 | 0 | 0 | 85 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 100 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 3 | 47 | N/A | 30 | 0 | 10 | 95 | N/A | 70 | 0 | 10 | 75 | N/A | 85 | 95 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 4 | 55 | N/A | 25 | 0 | 10 | 90 | N/A | 65 | 0 | 15 | 70 | N/A | 95 | 100 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 5 | 65 | N/A | 25 | 0 | 10 | 95 | N/A | 65 | 0 | 20 | 75 | N/A | 85 | 100 | 95 | 100 | 100 | 70 | 90 | 100 | 100 |
| 6 | 68 | N/A | 25 | 5 | 10 | 90 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 100 | 95 | 100 | 100 | 70 | 90 | 100 | 100 |
| 7 | 74 | N/A | 25 | 0 | 10 | 95 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 100 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 8 | 78 | N/A | 25 | 0 | 10 | 100 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 100 | 100 | 100 | 100 | 70 | 90 | 100 | 100 |
| 9 | 86 | N/A | 25 | 0 | 10 | 95 | N/A | 70 | 0 | 15 | 80 | N/A | 85 | 100 | 100 | 100 | 100 | 65 | 90 | 100 | 100 |
| 10 | 95 | N/A | 25 | 0 | 10 | 95 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 95 | 100 | 100 | 100 | 65 | 90 | 100 | 100 |
| 11 | 114 | N/A | 25 | 0 | 10 | 95 | N/A | 70 | 0 | 15 | 80 | N/A | 95 | 100 | 100 | 100 | 100 | 65 | 90 | 100 | 100 |
| 12 | 149 | N/A | 25 | 0 | 10 | 80 | N/A | 75 | 0 | 15 | 75 | N/A | 85 | 100 | 95 | 100 | 100 | 60 | 90 | 100 | 100 |
| 13 | 221 | N/A | 25 | 0 | 10 | 65 | N/A | 75 | 5 | 0 | 90 | N/A | 90 | 100 | 100 | 100 | 100 | 60 | 90 | 100 | 100 |
| 14 | 246 | N/A | 30 | 0 | 10 | 100 | N/A | 80 | 5 | 10 | 90 | N/A | 90 | 100 | 100 | 100 | 100 | 55 | 90 | 100 | 100 |
| 15 | 259 | N/A | 25 | 0 | 15 | 90 | N/A | 80 | 5 | 10 | 90 | N/A | 90 | 95 | 100 | 100 | 100 | 55 | 90 | 100 | 100 |

| Donor | | M5 | | | | | M6 | | | | | F1 | | | | | F2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 85 | 100 | 100 | 100 | 100 | 100 |
| 2 | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 95 | 100 | 100 | 100 | 100 | 100 |
| 3 | 47 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 95 | 100 | 100 | 100 | 100 | 100 |
| 4 | 55 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 95 | 100 | 100 | 100 | 100 | 100 |
| 5 | 65 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 85 | 65 | 95 | 75 | 100 | 100 | 100 | 100 | 100 |
| 6 | 68 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 90 | 100 | 100 | 100 | 100 | 100 |
| 7 | 74 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 90 | 100 | 100 | 100 | 100 | 100 |
| 8 | 78 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 90 | 100 | 100 | 100 | 100 | 100 |
| 9 | 86 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 90 | 100 | 100 | 100 | 100 | 100 |
| 10 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 95 | 95 | 100 | 100 | 100 | 100 | 100 |
| 11 | 114 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 85 | 95 | 90 | 100 | 100 | 100 | 100 | 100 |
| 12 | 149 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 90 | 100 | 100 | 100 | 100 | 100 |
| 13 | 221 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 80 | 90 | 70 | 100 | 100 | 100 | 100 | 100 |
| 14 | 246 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 85 | 100 | 100 | 100 | 100 | 100 |
| 15 | 259 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 90 | 90 | 80 | 100 | 100 | 100 | 100 | 100 |

| Donor | | F3 | | | | | F4 | | | | | F5 | | | | | mean all donors | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 80 | 83.2 | 96.4 |
| 2 | 6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 87.3 | 80 | 82.7 | 96.4 |
| 3 | 47 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 79.5 | 83.2 | 96.8 |
| 4 | 55 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 80 | 83.2 | 95.9 |
| 5 | 65 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 84.5 | 77.7 | 83.6 | 95 |
| 6 | 68 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 87.3 | 80.5 | 83.2 | 96.4 |
| 7 | 74 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 87.3 | 80 | 83.6 | 96.8 |
| 8 | 78 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 87.3 | 80 | 83.6 | 97.3 |
| 9 | 86 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 85.9 | 80 | 83.6 | 96.8 |
| 10 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 79.5 | 83.6 | 97.3 |
| 11 | 114 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 79.5 | 83.6 | 96.8 |
| 12 | 149 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 85.9 | 80 | 82.7 | 95 |
| 13 | 221 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.4 | 79.5 | 81.8 | 93.2 |
| 14 | 246 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.8 | 80.5 | 82.7 | 97.7 |
| 15 | 259 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 86.4 | 80 | 83.2 | 96.4 |

| Donor | | mean M1 and M2 | | | | | mean M3-6 F1-5 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CpG | nt | #1 | #2 | #3 | #4 | #5 | #1 | #2 | #3 | #4 | #5 |
| 1 | 1 | N/A | 50 | 0 | 12.5 | 87.5 | 100 | 95 | 97.8 | 98.9 | 98.3 |
| 2 | 6 | N/A | 47.5 | 0 | 7.5 | 82.5 | 100 | 96.1 | 97.8 | 99.4 | 99.4 |
| 3 | 47 | N/A | 50 | 0 | 10 | 85 | 100 | 95 | 97.2 | 99.4 | 99.4 |
| 4 | 55 | N/A | 45 | 0 | 12.5 | 80 | 100 | 96.1 | 97.8 | 98.9 | 99.4 |
| 5 | 65 | N/A | 45 | 0 | 15 | 85 | 100 | 93.3 | 95 | 98.9 | 97.2 |
| 6 | 68 | N/A | 47.5 | 2.5 | 12.5 | 85 | 100 | 96.1 | 97.8 | 98.9 | 98.9 |
| 7 | 74 | N/A | 47.5 | 0 | 12.5 | 87.5 | 100 | 96.1 | 97.8 | 99.4 | 98.9 |
| 8 | 78 | N/A | 47.5 | 0 | 12.5 | 90 | 100 | 96.1 | 97.8 | 99.4 | 98.9 |
| 9 | 86 | N/A | 47.5 | 0 | 12.5 | 87.5 | 100 | 94.4 | 97.8 | 99.4 | 100 |
| 10 | 95 | N/A | 47.5 | 0 | 12.5 | 87.5 | 100 | 95.6 | 97.2 | 99.4 | 99.4 |
| 11 | 114 | N/A | 47.5 | 0 | 12.5 | 87.5 | 100 | 95.6 | 97.2 | 99.4 | 98.9 |
| 12 | 149 | N/A | 50 | 0 | 12.5 | 77.5 | 100 | 93.9 | 97.8 | 98.3 | 98.9 |
| 13 | 221 | N/A | 50 | 2.5 | 5 | 77.5 | 100 | 94.4 | 96.7 | 98.9 | 96.7 |
| 14 | 246 | N/A | 55 | 2.5 | 10 | 95 | 100 | 93.9 | 97.8 | 98.9 | 98.3 |
| 15 | 259 | N/A | 52.5 | 2.5 | 12.5 | 90 | 100 | 93.9 | 97.2 | 98.9 | 97.8 |
| mean | | N/A | 48.7 | 0.7 | 11.5 | 85.7 | 100 | 95 | 97.4 | 99.1 | 98.7 |

| mean all donors | | | | |
|---|---|---|---|---|
| 100 | 86.6 | 79.8 | 83.2 | 96.3 |

| Foxp3 TSDR Top 700bp/500bp | | | | | | | | | | Foxp3 TSDR Top/Reverse Strands 700bp | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Donor | | M2 | | | | F2 | | | | M2 | | | | F1 | | | | M4 | | | | | | | |
| | subset | #3 | #3 | #5 | #5 | #2 | #2 | #5 | #5 | #3 | #3 | #5 | #5 | #3 | #3 | #5 | #5 | #2 | #2 | #4 | #4 | #5 | #5 |
| CpG | nt | T700 | T500 | T700 | T500 | T700 | T500 | T700 | T500 | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS |
| 1 | 1 | 0 | 0 | 100 | 90 | 10 | 20 | 100 | 90 | 0 | 0 | 100 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 2 | 6 | 10 | 0 | 100 | 100 | 0 | 20 | 90 | 80 | 0 | 0 | 100 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 3 | 47 | 0 | 0 | 100 | 90 | 10 | 20 | 100 | 100 | 0 | 0 | 80 | 100 | 40 | 100 | 90 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 4 | 55 | 0 | 0 | 100 | 100 | 10 | 20 | 100 | 100 | 0 | 0 | 100 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 5 | 65 | 0 | 0 | 100 | 100 | 0 | 20 | 100 | 100 | 0 | 0 | 90 | 100 | 60 | 100 | 80 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 6 | 68 | 0 | 0 | 100 | 90 | 0 | 20 | 100 | 100 | 0 | 0 | 100 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 7 | 74 | 0 | 0 | 100 | 100 | 0 | 20 | 100 | 100 | 10 | 0 | 30 | 100 | 20 | 100 | 70 | 100 | 0 | 100 | 10 | 100 | 90 | 100 |
| 8 | 78 | 0 | 0 | 100 | 100 | 0 | 20 | 100 | 100 | 0 | 0 | 100 | 100 | 70 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 9 | 86 | 20 | 0 | 100 | 100 | 0 | 20 | 100 | 80 | 0 | 0 | 100 | 100 | 80 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 10 | 95 | 0 | 0 | 100 | 100 | 0 | 20 | 90 | 100 | 0 | 0 | 90 | 100 | 70 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 11 | 114 | 0 | 0 | 100 | 100 | 10 | 20 | 100 | 100 | 0 | 0 | 100 | 100 | 60 | 100 | 90 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 12 | 149 | 0 | 0 | 90 | 90 | 20 | 0 | 60 | 60 | 0 | 0 | 100 | 100 | 60 | 100 | 60 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 13 | 221 | 0 | 0 | 90 | 70 | 10 | 20 | 70 | 40 | 0 | 0 | 100 | 100 | 60 | 100 | 50 | 100 | 0 | 100 | 0 | 100 | 80 | 100 |
| 14 | 246 | 0 | 0 | 100 | 100 | 40 | 20 | 90 | 100 | 0 | 0 | 100 | 100 | 70 | 100 | 90 | 100 | 0 | 100 | 0 | 100 | 100 | 100 |
| 15 | 259 | 0 | 0 | 100 | 100 | 20 | 20 | 100 | 100 | 10 | 0 | 100 | 100 | 70 | 100 | 80 | 100 | 0 | 100 | 0 | 100 | 90 | 100 |
| mean all CpGs | | 2 | 0 | 98.7 | 95.3 | 8.7 | 18.7 | 93.3 | 90 | 1.3 | 0 | 92.7 | 100 | 60 | 100 | 87.3 | 100 | 0 | 100 | 0 | 100 | 97.3 | 100 |

| Foxp3 TSDR Top/Reverse Strands 700bp | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Donor | | M6 | | | | | | F3 | | | | | | F4 | | | | | | | |
| | subset | #2 | #2 | #4 | #4 | #5 | #5 | #2 | #2 | #4 | #4 | #5 | #5 | #2 | #2 | #4 | #4 | #5 | #5 | | |
| CpG | nt | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | TS | RS | | |
| 1 | 1 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 2 | 6 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 90 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 3 | 47 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 90 | 100 | | |
| 4 | 55 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 90 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 5 | 65 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 6 | 68 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 90 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 7 | 74 | 0 | 100 | 50 | 100 | 100 | 100 | 20 | 100 | 30 | 100 | 80 | 100 | 50 | 100 | 70 | 100 | 50 | 100 | | |
| 8 | 78 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 9 | 86 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 90 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 10 | 95 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 90 | 100 | | |
| 11 | 114 | 0 | 100 | 70 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 50 | 100 | 100 | 100 | 100 | 100 | | |
| 12 | 149 | 0 | 100 | 70 | 100 | 90 | 100 | 0 | 100 | 0 | 100 | 80 | 100 | 0 | 100 | 50 | 100 | 80 | 100 | | |
| 13 | 221 | 0 | 100 | 60 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 80 | 100 | 40 | 100 | 100 | 100 | 70 | 100 | | |
| 14 | 246 | 0 | 100 | 70 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 40 | 100 | 100 | 100 | 100 | 100 | | |
| 15 | 259 | 0 | 100 | 70 | 100 | 100 | 100 | 0 | 100 | 0 | 100 | 100 | 100 | 40 | 100 | 100 | 100 | 90 | 100 | | |
| mean all CpGs | | 0 | 100 | 62 | 100 | 99.3 | 100 | 1.3 | 100 | 2 | 100 | 93.3 | 100 | 44.7 | 100 | 94.7 | 100 | 91.3 | 100 | | |

**Supplementary Table 3.** Methylation percentages for *CAMTA1*, *FUT7*, *FOXP3* enhancer, preTSDR, promoter and TSDR regions in five cell populations of donors M1-6 and F1-5. Methylation percentages for individual CpG sites were calculated based on the number of $^{m}$Cs in a total of 20 sequences unless otherwise stated. CpGs in each amplicon are numbered relative to the 5'-3' direction of the coding strand with CpG 1 denoted nucleotide (nt) position 1. The five cell subsets are: #1 ($CD34^{+}$), #2 ($CD45RA^{+}CD15s^{-}$), #3 ($CD45RA^{-}CD15s^{-}$), #4 ($CD45RA^{-}CD15s^{+}$) and #5 (Tcon). TS- top strand, RS – reverse strand

| **Human *FOXP3* TSDR** | | |
|---|---|---|
| **Reference** | **Based on** | **Strand** |
| (1) | Primers | Top |
| (2) | Primers | Reverse |
| (3) | Primers | Top |
| (4) | Primers | Reverse/Top |
| (5) | Primers | Top |
| (6) | Primers | Reverse |
| (7) | (1) | Top |
| (8) | (5) | Top |
| (9) | (5) | Top |
| (10) | (5) | Top |
| (11) | (5) | Top |
| (12) | (5) | Top |
| (13) | Primers | Reverse/Top |
| (14) | Primers | Reverse |
| (15) | Primers | Top |
| (16) | (5) | Top |
| (17) | (4) | Top |
| (18) | (5) | Top |
| (19) | Primers | Top |
| (20) | Primers | Top |
| (21) | Primers | Top |
| (22) | (1) | Top |
| (23) | Primers | Top |
| (24) | Primers | Reverse |
| (25) | (5) | Top |
| (26) | (5) | Top |
| (27) | EpigenDx | --- |
| (28) | (1) | Top |
| (29) | (1) | Top |
| (30) | Primers | Reverse |
| (31) | Primers | Reverse |
| (32) | (5) and primers | Top |
| (33) | (5) | Top |
| (34) | Primers | Top |
| (35) | (5) | Top |
| (36) | Primers | Top |
| **Murine *FOXP3* TSDR** | | |
| (37) | Primers | Reverse |
| (38) | (37) | Reverse |
| (39) | Primers | Top |
| (40) | Primers | Top |
| (41) | EpigenDx | --- |
| (42) | EpigenDx | --- |
| (43) | Primers | Reverse |
| (44) | Primers | Reverse |
| (45) | (40, 44) | Top, Reverse |
| (46) | (37) | Reverse |
| (47) | Primers | Top |
| (48) | Primers | Top |

| | | |
|---|---|---|
| (49) | Primers | Top |
| (50) | Primers | Reverse /Top |
| (51) | (43) | Reverse |
| (52) | (39), (43) | Top, Reverse |
| (53) | Primers | Top |
| (54) | Primers | Top |
| (55) | Primers | Top |
| **Human *FOXP3* Promoter** | | |
| (38) | Primers | Top |
| (56) | Primers | Top |
| (2) | Primers | Reverse |
| (3) | Primers | Top |
| (57) | Primers | Top |
| (6) | Primers | Reverse |
| (13) | Primers | Reverse |
| (19) | Primers | Top |
| (21) | Primers | Reverse |
| (25) | Primers | Top |
| (58) | Primers | Reverse |
| (59) | Primers | Top |
| (60) | Primers | Reverse |
| (61) | Primers | Top |

**Supplementary Table 4.** Studies that used human and murine *FOXP3* TSDR and promoter regions. Template strands used to detect methylation status of the two regions were identified using primer sequences supplied in the article or *via* reference (which then had primer sequence) used in Methodology section when primer sequences were not available

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