**Supplementary Table 1. Sequences used for bisulfite pyrosequencing.**

|  |  |
| --- | --- |
| **Gene** | **Sequence analyzed\*** |
| ***FOXP3* Sequence 1****CpG number**  | CRTAACAATTTCCCACAAACCAAACTA 1 |
| ***FOXP3* Sequence 2****CpG number**  | RACTTCCACACCRTACAACRTAATTTTTCTTCTCRATATA1 2 3 4  |
| ***FOXP3* Enhancer Sequence 1****CpG number**  | TGTTTYGAGTTTTTATYGTTGTGTTTYGTTTTYGT 1 2 3 4 |
| ***FOXP3* Enhancer Sequence 2****CpG number**  | TTYGTTGTYGTTYGYGTYGGGTYGTTTGGAGYGT 1 2 3 4 5 6 7 |
| ***RORC1* Sequence 1****CpG number** | TATTTTGGYGGGTGGAAATAGTTTTTATYGYGT 1 2 3  |
| ***RORC1* Sequence 2****CpG number** | ATTTTTTAAYGTTTTTTAYGATTGGTTGTT 1 2  |
| ***RORC1* Sequence 3****CpG number** | GTTAYGTGGGGYGTTTGTTATTTTATTTATTGTATTT 1 2  |
| ***RORC2* Sequence 1****CpG number** | TATTTTGGYGGGTGGAAATAGTTTTTATYGYGTGTGGTTGTYGT 1 2 3 4  |
| ***RORC2* Sequence 2****CpG number** | TTTTTTAAYGTTTTTTAYGATTGGTTGTTTTTTTTTTTATATT 1 2  |
| ***STAT3* Sequence 1****CpG number** | YGTYGTTYGTTTTYGGTATAYGYGTAGTTTYGGT1 2 3 4 5 6 7 |
| ***STAT5A* Sequence 1****CpG number** | GGYGTTTAGTTYGATTTTATTAAATTTTTTGGGTTTYGTG 1 2 3  |
|  | \*analyzed CpG sites are underlined and consecutively numbered  |

**Legend to supplementary Table 1.**

*FOXP3* promoter (human build hg 19 Chromosome X, 49121152-49121485 bp, len: 333) and *FOXP3* enhancer (Chromosome X, 49126597-49126750 bp, len: 153), *RORC1* (Chromosome 1, 151798543-151798859 bp, len: 316) and *RORC2* (Chromosome 1, 151798622-151798858 bp, len: 236), *STAT3* (Chromosome 17, 40540288-40540536 bp, len: 248) and *STAT5A* (Chromosome 17, 40439466-40439596 bp, len: 130) (ensemble releaser 15th February 2014).

PCR amplifications were performed with an initial denaturation step at 95°C for 5min, 40 cycles of 95°C for 30s, primer-specific annealing temperature of 60°C for 30s, 72 °C for 45s, and a final extension step at 72°C for 10min. The reaction mixture consisted of 2.5 µl 10x PCR buffer with MgCl2, 0.5 µl 10 mM dNTP mix, 1.25 µl of each forward and reverse primer, 0.2 µl (5 U/µl) Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 18.3 µl PCR-grade water, and 1 µl template DNA (75 ng).

**Supplementary Table 2. Primers used for bisulfite pyrosequencing.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Primer** | **Sequence (5’-3’)** | **Number of CpGs** |
| ***FOXP3*** | forward | Biotin-AGTTTGGTTTGTGGGAAATTGTT |  |
|  | reverse | ACCCTATTATCTCATTAATACCTCTCA |  |
|  | Sequence 1 | ATAAAAACAAAATTATTTTTAATA | 1 |
|  | Sequence 2 | AAATTATTAAAAAAAAAAAATCTAC | 4 |
| ***FOXP3* Enhancer** | forward | ATGAAGGGGAGGAGGAAG |  |
|  | reverse | Biotin-CCTCCAACTCCACCATAAC |  |
|  | Sequence 1 | GAGGAAGAGGAGGTT | 4 |
|  | Sequence 2 | GGGTTTTATTTGGTTTTTATATT | 7 |
| ***RORC1*** | forward | GGGGGTAGGAGGAGTAGAAAT |  |
|  | reverse | Biotin-CTAACTCTCTCCCCCAATACTTC |  |
|  | Sequence 1 | ATTTTGGTTTTTTAGTTAGAAT | 3 |
|  | Sequence 2 | GTATGTGGTTTTGGA | 2 |
|  | Sequence 3 | ATATTTTGTTTTAGGTTTAGATTG | 2 |
| ***RORC2*** | forward | GGGGTAGGAGGAGTAGAAA |  |
|  | reverse | Biotin-CCCACCCCCAAAAAATACAATAAATA |  |
|  | Sequence 1 | ATTTTGGTTTTTTAGTTAGAAT | 4 |
|  | Sequence 2 | GTATGTGGTTTTGGAA | 2 |
| ***STAT3*** | forward | GGTTGAAGGGGTTGTAAT |  |
|  | reverse | Biotin-CCCTTCACCTATTTCTCC |  |
|  | Sequence 1 | GGTTTGAGGGAGTTT | 7 |
| ***STAT5A*** | forward | GAAGTTGGGTAAAGGGGATGGA |  |
|  | reverse | Biotin-CAAACCTTACCACAACAATAAATATCC |  |
|  | Sequence 1 | GGTAAAGGGGATGGAA | 3 |

**Supplementary Table 3.** **Primers used for quantitative PCR.**

|  |  |  |
| --- | --- | --- |
| **Gene**  | **Forward sequence (5’-3’)** | **Reverse** |
| ***FOXP3*** | TCATCTGTGGCATCATCCGA | GGAACTCTGGGAATGTGCTG |
| ***RORC***  | TTCTCAAAGCAGGAGCAATGG | TGGGAGAAGTCAAAGATGGAG |
| ***STAT3*** | GGAACGAAGGGTACATCATGG | TCCACCCAAGTGAAAGTGAC |
| ***STAT5*** | TCACTGAAGAGGATCAAGCGT | GATGACAACCACAGGTAGGGA |
| ***CCR6*** | ATGAACCGATCCTGCCAGA | GAGAAGCCTGAGGACTTGT |
| ***IL-10*** | CCTGCCTAACATGCTTCGAG | CAACCCAGGTAACCCTTAAAGTC |
| ***IL-17*** | CTCATTGGTGTCACTGCTACTG | CACTTTGCCTCCCAGATCAC |
| ***2M*** | CCAGCAGAGAATGGAAAGTC | GATGCTGCTTACATGTCTCG |

**Legend to supplementary Table 3.**

Forkhead box protein 3 (*FOXP3*), RAR-related orphan receptor C (*RORC*), signal transducer and activator of transcription 3 (*STAT3*), signal transducer and activator of transcription 5 (*STAT5*), chemokine receptor 6 (*CCR6*), interleukin-10 (*IL-10*), interleukin-17 (*IL-17*), beta2-microglobulin (*2M).*

**Supplementary Table 4. Methylation status at each CpG position.**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **dcSSc** | **HC** | **p-value** |
|  | **male** | **female** | **male** | **female** | **male** | **female** |
| ***FOXP3* sequence 1 CpG1** | 65.38±4.47 (66.48; 58.07-69.89) | 68.69±4.41(69.89; 60.67-73.02) | 63.59±6.54 (59.79; 57.91-71.15) | 63.91±2.74 (62.45; 61.87-68.52) | 0.917 | 0.062 |
| ***FOXP3* sequence 2 CpG1** | 86.18±1.87 (85.93; 84.28-89.09 | 88.30±2.64 (89.04; 83.86-92.00) | 83.78±3.22 (83.00; 79.90-88.00) | 88.26±2.53 (88.88; 84.47-90.85) | 0.251 | 0.886 |
| ***FOXP3* sequence 2 CpG2** | 69.19±3.03 (68.63; 64.98-72.67) | 75.11±4.25 (74.93; 70.35-81.00) | 68.88±4.08 (67.00; 65.00-75.00) | 73.89±3.31 (72.53; 71.38-80.00) | 0.754 | 0.886 |
| ***FOXP3* sequence 2 CpG3** | 70.70±2.40 (71.34; 67.86-73.40) | 75.13±3.88 (74.52; 69.39-82.00) | 69.94±3.78 (70.00; 64.68-74.00) | 72.03±4.22 (70.85; 68.18-80.00) | 0.917 | 0.116 |
| ***FOXP3* sequence 2 CpG4** | 65.43±2.57 (64.77; 61.96-68.68) | 74.71±5.74 (74.68; 66.12-81.00) | 61.75±6.20 (64.00; 51.76-67.00) | 70.99±5.49 (71.85; 62.38-76.00) | 0.251 | 0.317 |
| ***FOXP3* enhancer sequence 1 CpG1** | 5.01±3.81 (2.56; 1.93-9.51) | 43.52±3.05 (42.91; 39.51-48.14) | 3.36±1.59 (2.47; 1.87-5.36) | 42.12±5.37 (42.93; 32.25-47.98) | 0.602 | 0.886 |
| ***FOXP3* enhancer sequence 1 CpG2** | 9.57±6.85 (5.30; 4.16-19.75) | 45.51±5.22 (44.26; 38.73-53.46) | 8.06±3.66 (6.42; 4.46-12.41) | 45.34±5.61 (44.66; 38.66-53.83) | 0.917 | 0.775 |
| ***FOXP3* enhancer sequence 1 CpG3** | 7.32±3.84 (4.96; 4.19-11.70) | 44.99±4.73 (42.70; 38.08-50.53) | 6.62±3.26 (5.15; 3.55-10.64) | 46.02±5.63 (47.63; 38.93-52.73) | 0.465 | 0.775 |
| ***FOXP3* enhancer sequence 1 CpG4** | 4.80±2.74 (3.52; 2.02-8.78) | 39.84±6.41 (41.06; 27.30-47.35) | 3.58±2.40 (2.06; 1.84-7.32) | 33.74±11.97(38.69; 18.18-44.61) | 0.347 | 0.475 |
| ***FOXP3* enhancer sequence 2 CpG1** | 4.17±3.24 (2.34; 1.32-9.01) | 35.56±3.30 (36.24; 30.57-40.63) | 3.83±3.14 (2.52; 0.92-7.39) | 34.53±12.19 (37.83; 13.74-48.01) | 0.754 | 0.475 |
| ***FOXP3* enhancer sequence 2 CpG2** | 7.19±3.23 (6.33; 3.40-12.11) | 41.00±4.17 (40.62; 34.06-46.61) | 6.65±4.43 (4.47; 1.94-12.27) | 39.05±11.26 (44.54; 19.04-47.85) | 0.754 | 0.668 |
| ***FOXP3* enhancer sequence 2 CpG3** | 5.28±3.89 (3.15; 2.37-11.34) | 40.57±3.66 (39.81; 36.13-44.78) | 5.65±4.40 (2.97; 1.65-11.28) | 34.36±9.55(37.94; 16.93-43.20) | 0.917 | 0.199 |
| ***FOXP3* enhancer sequence 2 CpG4** | 3.55±2.61 (1.85; 1.55-7.39) | 32.37±5.95 (35.32; 25.18-39.35) | 3.14±2.64 (1.47; 0.90-6.58) | 27.83±9.00 (29.28; 11.91-38.43) | 0.347 | 0.391 |
| ***FOXP3* enhancer sequence 2 CpG5** | 4.50±3.53 (2.55; 2.11-10.34) | 45.09±3.96 (45.52; 37.16-49.10) | 4.06±3.34 (1.94; 1.42-8.11) | 39.44±12.36 (44.88; 16.45-49.00) | 0.347 | 0.568 |
| ***FOXP3* enhancer sequence 2 CpG6** | 4.08±2.64 (2.54; 2.24-8.44) | 40.43±3.35 (41.08; 34.06-43.90) | 4.64±3.58 (2.54; 1.36-8.89) | 36.40±11.25 (41.58; 15.27-44.28) | 1.000 | 1.000 |
| ***FOXP3* enhancer sequence 2 CpG7** | 6.08±3.17 (4.16; 3.55-11.11) | 37.33±5.13 (39.76; 28.98-42.24) | 6.33±4.25 (4.94; 2.24-11.99) | 32.13±8.56 (36.12; 15.31-37.17) | 0.917 | 0.199 |
| ***STAT3*****CpG1** | 2.32±1.87 (1.84; 0.00-6.42) | 1.71±1.95 (1.53; 0.00-5.75) | 0.402 |
| ***STAT3*****CpG2** | 3.47±2.28 (2.93; 1.35-8.23) | 3.53±2.96 (2.77; 0.00-9.69) | 0.951 |
| ***STAT3*****CpG3** | 4.58±3.49 (3.35; 1.76-14.01) | 5.09±3.34 (3.40; 2.25-11.51) | 0.538 |
| ***STAT3*****CpG4** | 4.48±2.91 (3.16; 2.10-10.29) | 3.93±3.41 (2.39; 1.32-10.72) | 0.268 |
| ***STAT3*****CpG5** | 7.35±4.14 (6.74; 3.08-16.27) | 7.27±4.04 (5.41; 3.08-14.92) | 0.926 |
| ***STAT3*****CpG6** | 2.52±1.66 (2.21; 0.00-6.45) | 2.68±1.89 (2.44; 0.00-7.05) | 0.782 |
| ***STAT3*****CpG7** | 7.78±6.89 (4.52; 2.39-26.46) | 6.78±4.68 (4.59; 2.96-18.81) | 0.854 |
| ***STAT5A*****CpG1** | 6.31±1.99 (6.05; 3.68-11.63) | 9.86±1.11 (9.66; 7.34-11.38) | 0.001 |
| ***STAT5A*****CpG2** | 4.40±2.00 (3.76; 2.15-8.07) | 4.39±1.39 (4.80; 2.25-6.23) | 0.580 |
| ***STAT5A*****CpG3** | 13.33±9.75 (6.79; 7.22-27.25) | 12.80±4.96 (9.75; 6.59-18.89) | 0.758 |
| ***RORC1* sequence 1****CpG1** | 43.07±5.78 (42.32; 31.35-52.47) | 34.30±4.85 (36.30; 23.20-39.21) | 0.001 |
| ***RORC1* sequence 1****CpG2** | 43.07±10.43 (43.40; 26.21-62.96) | 32.96±6.52 (35.35; 22.91-42.52) | 0.027 |
| ***RORC1* sequence 1****CpG3** | 24.99±5.10 (24.54; 17.92-33.54) | 17.47±3.67 (15.59; 13.82-23.91) | 0.001 |
| ***RORC1* sequence 2****CpG1** | 28.06±5.08 (28.02; 18.43-34.51) | 20.97±4.15 (20.76; 14.65-27.11) | 0.004 |
| ***RORC1* sequence 2****CpG2** | 20.71±3.89 (21.08; 12.67-25.64) | 15.30±2.51 (14.39; 10.44-18.86) | 0.003 |
| ***RORC1* sequence 3****CpG1** | 24.35±5.28 (24.51; 17.60-37.57) | 19.72±6.28 (19.47; 11.89-36.11) | 0.016 |
| ***RORC1* sequence 3****CpG2** | 53.74±5.32 (51.51; 46.78-62.97) | 44.03±5.41 (46.11; 32.24-49.69) | 0.0001 |
| ***RORC2* sequence 1****CpG1** | 44.91±6.39 (34.75-55.39) | 36.54±3.89 (36.01; 30.31-43.67) | 0.005 |
| ***RORC2* sequence 1****CpG2** | 45.55±9.55 (47.79; 28.58-58.30) | 35.49±6.90 (35.27; 26.17-47.96) | 0.012 |
| ***RORC2* sequence 1****CpG3** | 26.66±5.58 (25.97; 18.45-35.69) | 20.12±3.88 (19.19; 13.25-26.14) | 0.006 |
| ***RORC2* sequence 1****CpG4** | 43.38±6.81 (41.89; 32.26-54.29) | 36.52±5.19 (35.11; 28.57-45.84) | 0.019 |
| ***RORC2* sequence 2****CpG1** | 30.00±5.72 (32.27; 20.77-37.47) | 25.13±4.50 (26.10; 17.71-31.20) | 0.029 |
| ***RORC2* sequence 2****CpG2** | 21.43±3.18 (21.93; 16.91-26.10) | 16.21±3.06 (15.46; 11.77-20.45) | 0.003 |