

**Table S4.** Validation of the *tp*TAIL-PCR using different experimental protocols\*

| Patient** | Cell source | Technique          | Primers found | Clone | Amplicon sequence (nts) | kDNA/ host DNA (e-value)  |
|-----------|-------------|--------------------|---------------|-------|-------------------------|---|
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 5     | kDNA (333)              | <a href="#">emb AJ747981.1 </a> Trypanosoma cruzi kinetoplast minicircle, Expect = 3e-21  |
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-5    | 39    | kDNA (64)               | <a href="#">emb AJ747986.1 </a> Trypanosoma cruzi kinetoplast minicircle, Expect = 1e-21  |
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 79    | kDNA (286)              | <a href="#">gb EF128032.1 </a> Homo sapiens clone P2-13 trypanosome minicircle DNA integration region, Expect = 7e-29   |
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 89    | kDNA (286)              | <a href="#">gb AF242562.1 </a> Trypanosoma cruzi clone FGz 8-3 kinetoplast minicircle sequence, Expect = 2e-10  |
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 104   | kDNA (502)              | <a href="#">gb AY584193.1 </a> Homo sapiens clone G10 Trypanosoma cruzi infected macrophage p15 gene disrupted by LINE-1 element and Trypanosoma cruzi minicircle kinetoplast sequence, Expect = 1e-135 |
| C1        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 106   | kDNA (224)              | <a href="#">gb EF128032.1 </a> Homo sapiens clone P2-13 trypanosome minicircle DNA integration region, Expect = 6e-08   |
| C2        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-2    | 114   | kDNA (492)              | <a href="#">gb AF399842.1 AF399842</a> Trypanosoma cruzi clone 5 integrated kinetoplast, Expect = 1e-46   |
| C2        | Somatic     | <i>pf</i> TAIL-PCR | 67rev/L1-5    | 123   | kDNA (101)              | <a href="#">gb AY584193.1 </a> Homo sapiens clone G10 Trypanosoma cruzi infected macrophage p15 gene disrupted by LINE-1 element and Trypanosoma cruzi minicircle, Expect = 7e-                         |

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|    |         |                    |             |     |            | 27   |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-5  | 140 | kDNA (388) | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, clone KY-13, Expect = 1e-45  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-2  | 156 | kDNA (291) | <a href="#">gb AF241157.1 </a> Trypanosoma cruzi clone FGz 71 kinetoplast minicircle DNA, Expect = 3e-39   |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-2  | 162 | kDNA (263) | <a href="#">gb AF242563.1 </a> Trypanosoma cruzi clone FGz 8-4 kinetoplast minicircle sequence, Expect = 4e-08<br><a href="#">gb AC079399.5 </a> Homo sapiens BAC clone RP11-414P19 from 4, Expect = 0.0 |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/67rev | 172 | kDNA (454) | <a href="#">emb X04680.1 MITCMNC</a> Trypanosoma cruzi minicircle DNA, Expect = 2e-42  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-5  | 176 | kDNA (364) | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, clone y01 cst 4<br>Expect = 2e-49   |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-5  | 199 | kDNA (341) | <a href="#">gb M19187.1 TRBKPMCN</a> T.cruzi kinetoplast minicircle DNA, clone y01 cst 3, Expect = 8e-48   |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-2  | 250 | kDNA (130) | <a href="#">gb AF399842.1 AF399842</a> Trypanosoma cruzi clone 5 integrated kinetoplast minicircle DNA, Expect = 3e-10   |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-2  | 256 | kDNA (100) | <a href="#">gb M19185.1 TRBKPMCL</a> T.cruzi kinetoplast minicircle DNA, Expect = 3e-25  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-2  | 264 | kDNA (418) | <a href="#">emb X04680.1 MITCMNC</a> Trypanosoma cruzi minicircle DNA, Expect = 1e-34  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/67rev | 265 | kDNA (232) | <a href="#">gb U07846.1 TCU07846</a> Trypanosoma cruzi Y kinetoplast minicircle sequence, Expect = 4e-17   |
| C2 | Somatic | <i>pf</i> TAIL-    | 67rev/L1-2  | 266 | kDNA (548) | <a href="#">gb M19187.1 TRBKPMCN</a> T.cruzi kinetoplast   |

|    |         |                    |             |     |  |   |
|----|---------|--------------------|-------------|-----|--|---|
|    |         | PCR                |             |     |  | minicircle DNA, clone y01 cst 3, 2e-43  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/67rev | 274 | kDNA (625)                                 | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, Expect = 5e-21  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-5  | 275 | kDNA (388)                                 | <a href="#">emb AJ747993.1 </a> Trypanosoma cruzi kinetoplast minicircle, lineage IIb, Expect = 3e-104  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/67rev | 280 | kDNA (1342)                                | <a href="#">emb AJ747993.1 </a> Trypanosoma cruzi kinetoplast minicircle, lineage IIb, Expect = 4e-108  |
| C2 | Somatic | <i>pf</i> TAIL-PCR | 67rev/L1-5  | 293 | kDNA (694)                                 | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, Expect = 1e-54  |
| 16 | Somatic | PCR                | 67rev/67rev | 73  | kDNA (1-295)<br>Host DNA (278-914)         | <a href="#">gb U07846.1 TCU07846</a> Trypanosoma cruzi Y kinetoplast minicircle sequence, Expect = 6e-08<br><a href="#">gb AC007741.4 </a> Homo sapiens BAC clone RP11-340F16 from 2, Expect = 0.0  |
| 16 | Somatic | PCR                | 67rev/L1-5  | 85  | kDNA (360)                                 | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, Expect = 2e-42   |
| 16 | Somatic | PCR                | 67rev/L1-5  | 88  | kDNA (1-387)<br>Host DNA (373-465)         | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, Expect = 9e-48<br><a href="#">emb AL732374.14 </a> Human DNA sequence from clone RP13-444K19 on chromosome X, Expect = 8e-36   |
| 16 | Somatic | PCR                | 67rev/67rev | 92  | kDNA (1-295/488-773)<br>Host DNA (284-499) | <a href="#">gb U07846.1 TCU07846</a> Trypanosoma cruzi Y kinetoplast minicircle sequence, Expect = 9e-11<br><a href="#">gb AC084364.20 </a> Homo sapiens 12q BAC RP11-626I20, Expect = 4e-42 / <a href="#">gb AC012596.4 </a> Homo sapiens BAC clone CTD-2523K17 from 7, Expect = 4e-48 |
| 27 | Somatic | PCR                | 67rev/L1-5  | 32  | kDNA (360)                                 | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, Expect = 4e-45   |
| 50 | Somatic | PCR                | 67rev/67rev | 115 | kDNA (328)                                 | <a href="#">gb AY289115.1 </a> Trypanosoma cruzi clone 8-13 integrated kinetoplast minicircle,  |

|    |          |     |             |       |                 |  |
|----|----------|-----|-------------|-------|-----------------|--|
|    |          |     |             |       |                 | Expect = 9e-08   |
| 50 | Somatic  | PCR | 67rev/L1-5  | 120   | kDNA (360)      | gb M19188.1 TRBKPMCO T.cruzi kinetoplast minicircle DNA, Expect = 6e-49  |
| 50 | Somatic  | PCR | L1-5/L1-5   | 131   | Host DNA (1589) | emb AL162420.13  Human DNA sequence from clone RP11-380P16 on chromosome 9, Contains the IFNA17 gene for interferon, Expect = 0.0  |
| 55 | Somatic  | PCR | 67rev/67rev | 156   | kDNA (328)      | gb AY289115.1  Trypanosoma cruzi clone 8-13 integrated kinetoplast minicircle, Expect = 9e-08  |
| 55 | Somatic  | PCR | 67rev/L1-5  | 161   | kDNA (380)      | emb AJ748036.1  Trypanosoma cruzi kinetoplast minicircle, Expect = 3e-135  |
| 55 | Somatic  | PCR | 67rev/L1-5  | 168   | kDNA (360)      | gb M19188.1 TRBKPMCO T.cruzi kinetoplast minicircle DNA, Expect = 6e-49  |
| 56 | Somatic  | PCR | 67rev/67rev | 173   | kDNA (328)      | gb AY289115.1  Trypanosoma cruzi clone 8-13 integrated kinetoplast minicircle, Expect = 9e-08  |
| 59 | Somatic  | PCR | 67rev/L1-5  | 188   | kDNA (380)      | emb AJ748036.1  Trypanosoma cruzi kinetoplast minicircle, Expect = 3e-135  |
| 59 | Somatic  | PCR | 67rev/L1-5  | 198   | Host DNA (165)  | emb AL732374.14  <span style="background-color: #d9ead3; border: 1px solid #d9ead3; padding: 0 2px;">D</span> Human DNA sequence from clone RP13-444K19 on chromosome X, Expect = 1e-46                                      |
| 6  | Germline | PCR | 67rev/L1-5  | E-247 | kDNA (104)      | emb X04680.1 MITCMNC Trypanosoma cruzi minicircle DNA, Expect = 6e-22  |
| 6  | Germline | PCR | L1-5/L1-5   | E-249 | Host DNA (1610) | emb AL162420.13  <span style="background-color: #d9ead3; border: 1px solid #d9ead3; padding: 0 2px;">D</span> Human DNA sequence from clone RP11-380P16 on chromosome 9 Contains the IFNA17 gene for interferon, Expect= 0.0 |
| 6  | Germline | PCR | 67rev/L1-5  | E-250 | kDNA (358)      | gb M19185.1 TRBKPMCL T.cruzi kinetoplast minicircle DNA, clone y01 cst 1, Expect= 1e-45  |
| 25 | Germline | PCR | 67rev/67rev | E-254 | kDNA (232)      | emb AJ747996.1  Trypanosoma cruzi kinetoplast minicircle, lineage IIb, strain Tu18 cl2, isolate 02mp37, Expect = 4e-17   |
| 25 | Germline | PCR | 67rev/L1-5  | E-256 | kDNA (369)      | gb M19188.1 TRBKPMCO T.cruzi kinetoplast minicircle DNA, clone y01 cst 4, Expect = 1e-39   |

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| 25 | Germline | PCR | 67rev/L1-5 | E-258 | kDNA (371)                         | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, clone KY-13, Expect = 9e-167   |
| 34 | Germline | PCR | 67rev/L1-5 | E-259 | kDNA (81)                          | <a href="#">emb X04680.1 MITCMNC</a> Trypanosoma cruzi minicircle DNA, Expect = 2e-21  |
| 34 | Germline | PCR | L1-5/L1-5  | E-261 | Host DNA (1610)                    | <a href="#">emb AL162420.13 </a> Human DNA sequence from clone RP11-380P16 on chromosome 9 Contains the IFNA17 gene for interferon, Expect= 0.0  |
| 71 | Germline | PCR | 67rev/L1-5 | E-262 | kDNA (100)                         | <a href="#">emb X04680.1 MITCMNC</a> Trypanosoma cruzi minicircle DNA, Expect = 5e-23  |
| 71 | Germline | PCR | 67rev/L1-5 | E-263 | kDNA (371)                         | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, clone KY-13, Expect = 1e-164   |
| 71 | Germline | PCR | 67rev/L1-5 | E-264 | kDNA (1-380)<br>Host DNA (381-530) | <a href="#">emb AJ748036.1 </a> Trypanosoma cruzi kinetoplast minicircle, lineage IIe, strain CL Brener, isolate JP726, Expect = 4e-135<br><a href="#">emb AL732374.14 </a> Human DNA sequence from clone RP13-444K19 on chromosome X Contains a mitochondrial ribosomal protein S18C (MRPS18C) pseudogene, the 3' end of the PHF8 gene for PHD finger protein 8 and a CpG island, complete sequence, Expect = 1e-52   |
| 71 | Germline | PCR | 67rev/L1-5 | E-267 | kDNA (388 bp)                      | <a href="#">gb M18814.1 TRBKPMCAA</a> T.cruzi kinetoplast minicircle DNA, clone KY-13, Expect = 1e-45  |
| 71 | Germline | PCR | 67rev/L1-5 | E-269 | kDNA (85 bp)                       | <a href="#">emb X04680.1 MITCMNC</a> Trypanosoma cruzi minicircle DNA, Expect = 1e-22  |
| 71 | Germline | PCR | 67rev/L1-5 | E-272 | Host DNA (1-93)<br>kDNA (94-141)   | <a href="#">gb U07846.1 TCU07846</a> Trypanosoma cruzi Y kinetoplast minicircle sequence, clone M369r, orf, complete cds, Expect = 2e-15<br><a href="#">emb AL732374.14 </a>  Human DNA sequence from clone RP13-444K19 on chromosome X Contains a mitochondrial ribosomal protein S18C (MRPS18C) pseudogene, the 3' end of the PHF8 gene for PHD finger protein 8 and a CpG island, Expect = 2e-36 |

|    |          |     |            |       |                                    |  |
|----|----------|-----|------------|-------|------------------------------------|--|
| 81 | Germline | PCR | 67rev/L1-5 | E-279 | kDNA (1-263)<br>Host DNA (264-326) | <a href="#">gb AC233981.2 </a> Homo sapiens BAC clone RP11-620C18 from chromosome x, Expect = 4e-25  |
| 81 | Germline | PCR | 67rev/L1-5 | E-280 | kDNA (1-387)<br>Host DNA (388-465) | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, clone y01 cst 4, Expect =9e-48<br><a href="#">emb AL732374.14 </a> Human DNA sequence from clone RP13-444K19 on chromosome X Contains a mitochondrial ribosomal protein S18C (MRPS18C) pseudogene, the 3' end of the PHF8 gene for PHD finger protein 8 and a CpG island, Expect =3e-34 |
| 81 | Germline | PCR | 67rev/L1-5 | E-282 | kDNA (387 bp)                      | <a href="#">gb M19188.1 TRBKPMCO</a> T.cruzi kinetoplast minicircle DNA, clone y01 cst 4, Expect = 4e-45   |

\* A mix of wild-type *T. cruzi* kDNA with DNA from two control individuals ( C1 and C2) born in Europe were used as templates for the *tp*TAIL-PCR amplifications. This procedure aimed at the specificity of the *tp*TAIL-PCR used to show the kDNA integrations in the population belonging to five studied families.

\*\* The blood mononuclear cells or the germline cells from the studied families patients showing the kDNA positive exams were used, and the results obtained with the traditional PCR technique can be compared with those obtained with the modified *tp*TAIL-PCR used in the study.