# Supplementary information

## A child with perinatal HIV infection and long-term sustained virological control following antiretroviral treatment cessation

Violari *et al*.

# Supplementary Figures

SEQ  child: 1372 bp;
Composition  506  A; 274  C; 324  G; 268  T; 0 OTHER
Percentage:  37%  A; 20%  C; 24%  G; 20%  T; 0%OTHER

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Molecular Weight (kDa): ssDNA: 425.63    dsDNA: 845.8
ORIGIN
903     CAGGGAGCTG GAAAGATTTG CACTTAACCC TGGTCTTTTA GAGACACCAG AAGGCTGTAG
963     ACAAATAATG ACACAGCTAC AACCAGCTCT TCAGACAGGA ACAGAGGAAC TTAAATCATT
1023    ATTTAACACA GTAGCAACGC TCTATTGTGT ACATGCACAG ATAGAGGTAC GAGACACCAA
1083    GGAAGCCTTA GACAGGATAG AGGAAGAACA AAGCAAAAGT CAGCAAAAAG CACAGCAAGC
1143    AGAAATGGCT GATAGAGGAA AGATCAGTCA AAATTATCCT ATAGTGCAGA ATATTCAAGG
1203    GCAAATGGTA CACCAGCCCA TATCACCAAG AACTTTGAAT GCATGGGTAA AAGTAATAGA
1263    GGAGAAGGCC TTCAGCCCAG AAGTGATACC CATGTTTACA GCATTATCAG AAGGAGCCAC
1323    CCCACAAGAT TTAAACACCA TGTTAAATAC AATAGGGGGA CATCAAGCAG CCATGCAAAT
1383    GTTAAAAGAT ACCATTAATG AGGAAGCTGC AGAATGGGAT AGGATACATC CAGTACATGC
1443    TGGACCTGTT GCACCAGGCC AAATGAGAGA ACCAAGGGGA AGTGACATAG CAGGAACTAC
1503    TAGTACCCTT CAAGAACAAA TAGCATGGAT AACAGGTAAC CCACCTATTC CAGTAGGAGA
1563    CATCTATAAG AGATGGATAA TTCTGGGGTT AAATAAAATA GTAAGAATGT ATAGCCCTGT
1623    CAGCATTTTG GACATAAGAC AAGGGCCAAA AGAACCTTTT AGAGACTATG TAGATCGGTT
1683    CTTTAAAACT TTAAGAGCTG AACAAGCTAC ACAAGAAGTA AAAAATTGGA TGACAGACAC
1743    ATTGTTAGTC CAAAATGCAA ACCCAGATTG CAAGACCATT TTAAGAGCAT TAGGACCAGG
1803    GGCTACATTA GAAGAAATGA TGACAGCATG TCAGGGAGTG GGAGGACCTA GCCACAAAGC
1863    AAGAGTTTTG GCTGAGGCAA TGAGTCAAGC AGGCAGTGCA GGCATAATGA TGCAGAGAAG
1923    CAATTTTAAA GGCCCTAGAA AAATTGTTAA ATGTTTCAAC TGTGGCAAGG AAGGGCACAT
1983    AGCTAGGAAT TGCAAGGCTC CCAGGAAAAA AGGCTGTTGG AAATGTGGAA GGGAAGGACA
2043    CCAAATGAAA GACTGCACTG AAAGGCAGGC TAATTTTTTA GGGAAAATTT GGCCTTCCCT
2103    CAAGGGGAGG CCAGGGAATT TCCTTCAGAG CAGACCAGAA CCAACAGCCC CACCAGCAGA
2163    GAGCTTCAGG TTCGAGGAGA CAACCCCCAC TCCGAAACAG GAGTCGAGAG ACAAGGAACC
2223    ACCCTTAACT GCCCTCAAAT CACTCTTTGG CAGCGACCCC TTGTTACAAT AA
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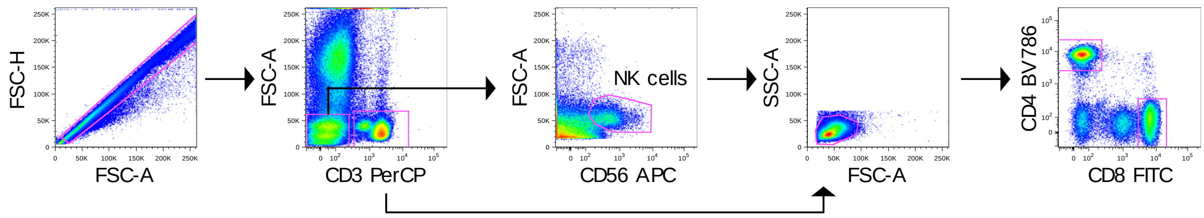
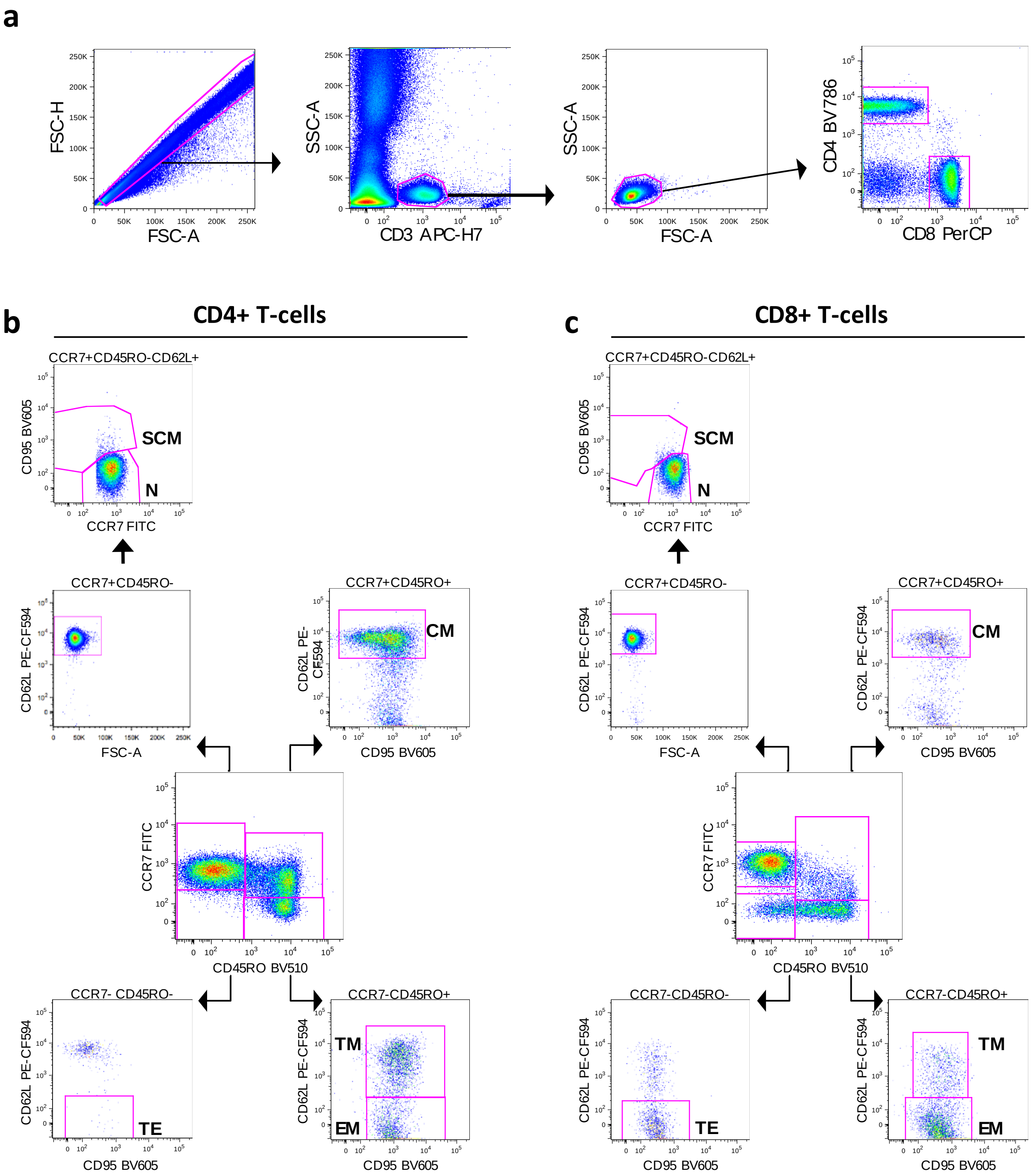
**Supplementary Figure 1.** *Consensus partial gag-pol sequence for the Case.* A consensus HIV-1 proviral partial *gag-pol* nucleotide sequence (HXB2 numbering nt 903-2292) was derived from alignment of 20 cloned sequences for the child (Genbank accession numbers MH789553-MH789572). Sequence denotes partial *gag* (903-2292) and partial *pol* (nt 2085-2292). Further breakdown of coding sequences includes partial *gag* p17 (nt 903-1185, aa 39-132), *gag* p24 (nt 1186-1878, aa 1-231), *gag* p2 1879-1920, aa 1-14), *gag* p7 (nt 1921-2085, aa 1-55), *gag* p1 (nt 2086-2133, aa 1-16), *gag* p6 (nt 2134-2292, aa 1-53) and partial protease sequence (nt 2253-2292, aa 1-14). Numbering according to the Los Alamos HIV Sequence database, www.hiv.lanl.gov

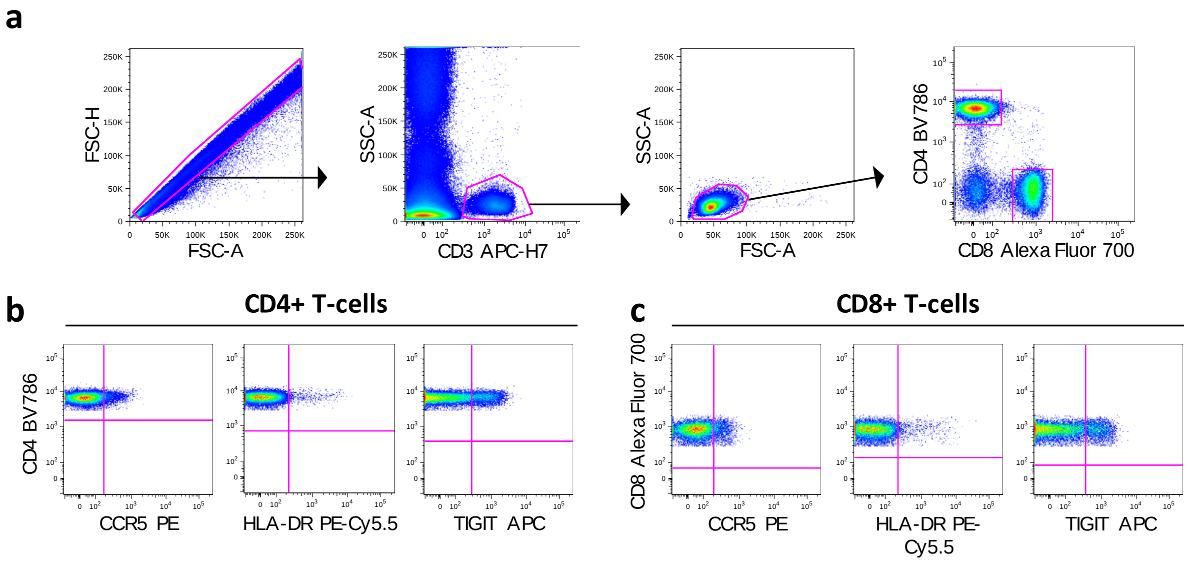



**Supplementary Figure 2.** *Gating strategy for the identification of CD4+ and CD8+ T-cells and NK cells for the intracellular cytokine assay (manuscript Fig. 3c, d).* Singlets were identified using FSC-H against FSC-A followed by gating on CD3+ and CD3- cells. CD3+ T-cells were further gated on low SSC-A vs low FSC-A (small lymphocytes) and then on CD4+ and CD8+ T-cells. NK cells were identified by gating CD3- CD56+ cells.
Antibody staining panel: CD3 PerCP, CD4 BV786, CD8 FITC, CD56 APC, IFN-γ PE, IL-2 PE.



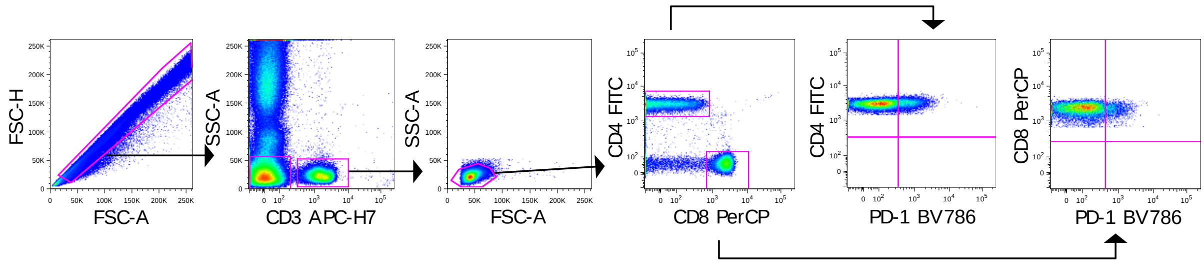

**Supplementary Figure 3.** *Gating strategy used to immunophenotype memory T-cell subsets (manuscript Fig.5b)*. Representative flow cytometry plots are from the Case. **a** Singlets were identified followed by gating on CD3+ lymphocytes. CD3+ T-cells were further gated on low SSC-A and FSC-A (small lymphocytes) and then on CD4+ or CD8+ T-cells. **b** Gating strategy for the determination of CD4+T-cell subsets: Naïve (N): CCR7+CD45RO-CD95-; Stem cell Memory (SCM): CCR7+CD45RO-CD95+; Central Memory (CM): CCR7+CD45RO+CD62L+; Transitional Memory (TM): CCR7-CD45RO+CD62L+; Effector Memory (EM): CCR7-CD45RO+CD62L- and Terminal Effector (TE): CCR7-CD45RO-CD62L-. **c** Gating strategy for determination of CD8+ T-cell subsets is similar to that for CD4+ T-cells (**b**). For the low frequency $T_{SCM}$ subset, the gating strategy outlined by Lugli *et al*[1] was utilized: the slightly lower level of CCR7 expression on $T_{CSM}$ compared to $T_N$ was utilized to provide a clear separation of positive and negative CD95 cells. A diagonal gate was drawn on the bivariate plot of CD95 versus CCR7 on the total T cell population (not shown) and then copied into the same bivariate plot after gating for CD45RO, CCR7 and CD62L.

Antibody staining panel: CD3 APC-H7, CD4 BV786, CD8 PerCP, CD45RO BV510, CCR7 (CD197) FITC, CD95 BV605, CD62L PE-CF594.
Immunophenotyping data (Supplementary Table 3).




**Supplementary Figure 4.** *Gating strategy for determining CCR5, HLA-DR and TIGIT expression on CD4+ and CD8+ T-cells (manuscript Fig. 5c)*. **a** Singlets were identified followed by gating on CD3+ lymphocytes. CD3+ T-cells were further gated on low SSC-A and FSC-A (small lymphocytes) and then on CD4+ or CD8+ T-cells. The expression of CCR5, HLA-DR and TIGIT on (**b**) CD4+ T-cells and (**c**) CD8+ T-cells are shown for the Case, respectively. Antibody staining panel: CD3 APC-H7, CD4 BV786, CD8 Alexa Fluor® 700, CCR5 PE, HLA-DR PE-Cy5.5, TIGIT APC.

Immunophenotyping data (Supplementary Table 3).




**Supplementary Figure 5.** *Gating strategy for determining PD-1 expression on CD4+ and CD8+ T-cells (manuscript Fig.5c)*. Singlets were identified followed by gating on CD3+ lymphocytes. CD3+ T-cells were further gated on low SSC-A and FSC-A (small lymphocytes) and then on CD4+ or CD8+ T-cells. PD-1 expressing CD4+ and CD8+ T-cells are shown for the Case.

Antibody staining panel: CD3 APC-H7, CD4 FITC, CD8 PerCP, CD279 (PD-1) BV786. Immunophenotyping data (Supplementary Table 3).

## Supplementary Tables

**Supplementary Table 1.** Longitudinal viral loads and CD4 T-cell counts and percentages of the Case (Fig.1a-c).

| Phase | Study Week | Age (weeks) | Age (years) | HAART | CD4 count | CD4 percentage | Viral Load | Log VL |
|---|---|---|---|---|---|---|---|---|
| Screening | -4.1 | 5.57 | 0.11 | No HAART | 3656 | 34.1 | >750, 000 | 5.88 |
| Screening | -1.1 | 8.57 | 0.16 | No HAART | 3708 | 41 | 151, 000 | 5.18 |
| Baseline | 0.0 | 9.71 | 0.19 | AZT+3TC+LPV/r | 2249 | 41.6 | not done | not done |
| Primary | 4.0 | 13.71 | 0.26 | AZT+3TC+LPV/r | 3583 | 33.9 | 915 | 2.96 |
| Primary | 12.0 | 21.71 | 0.42 | AZT+3TC+LPV/r | 2644 | 46.1 | 335 | 2.53 |
| Primary | 24.0 | 33.71 | 0.65 | AZT+3TC+LPV/r | 1976 | 42.8 | <50 | 1.70 |
| Primary | 32.0 | 41.71 | 0.80 | AZT+3TC+LPV/r | 2867 | 43.1 | 20 | 1.30 |
| Primary/Interruption Date | 40.0 | 49.71 | 0.95 | AZT+3TC+LPV/r | 1968 | 40.9 | <20 | 1.30 |
| Interruption | 48.0 | 57.71 | 1.11 | No HAART | 1857 | 42.7 | <20 | 1.30 |
| Interruption | 60.0 | 69.71 | 1.34 | No HAART | 2543 | 41 | <20 | 1.30 |
| Interruption | 72.4 | 82.14 | 1.57 | No HAART | 2143 | 38.1 | <20 | 1.30 |
| Interruption | 84.0 | 93.71 | 1.80 | No HAART | 1935 | 47 | <20 | 1.30 |
| Interruption | 96.0 | 105.71 | 2.03 | No HAART | 1492 | 40.4 | <400 | 1.30 |
| Interruption | 107.0 | 116.71 | 2.24 | No HAART | 1618 | 45.9 | <400 | 1.30 |
| Interruption | 116.0 | 125.71 | 2.41 | No HAART | 1390 | 43.8 | <20 | 1.30 |
| Interruption | 128.0 | 137.71 | 2.64 | No HAART | 1551 | 40.1 | <20 | 1.30 |
| Interruption | 139.7 | 149.43 | 2.86 | No HAART | 1447 | 38 | <20 | 1.30 |
| Interruption | 152.0 | 161.71 | 3.10 | No HAART | 1349 | 46.6 | not done | not done |
| Interruption | 162.7 | 172.43 | 3.30 | No HAART | 1440 | 40.8 | <20 | 1.30 |
| Interruption | 176.0 | 185.71 | 3.56 | No HAART | 1131 | 30.8 | <20 | 1.30 |
| Interruption/Final CHER Visit | 188.4 | 198.14 | 3.80 | No HAART | 1058 | 32.93 | <20 | 1.30 |
| Interruption/Post CHER Visit | 200.0 | 209.71 | 4.02 | No HAART | 1210 | 39.5 | not done | not done |
| Interruption/Post CHER Visit | 212.0 | 221.71 | 4.25 | No HAART | 1315 | 38.9 | not done | not done |
| Interruption/Post CHER Visit | 236.0 | 245.71 | 4.71 | No HAART | 1234 | 38.9 | not done | not done |
| Interruption/Post CHER Visit | 265.4 | 275.14 | 5.27 | No HAART | 1578 | 41.9 | not done | not done |
| Interruption/Post CHER Visit | 289.6 | 299.29 | 5.74 | No HAART | 1700 | 44.6 | not done | not done |
| Interruption/Post CHER Visit | 321.7 | 331.43 | 6.35 | No HAART | 1080 | 39.6 | <20 | 1.30 |
| Interruption/Post CHER Visit | 353.0 | 362.71 | 6.95 | No HAART | 1028 | 35.1 | <20 | 1.30 |
| Interruption/Post CHER Visit | 354.4 | 364.14 | 6.98 | No HAART | 1028 | 35.1 | <20 | 1.30 |
| Interruption/Post CHER Visit | 379.4 | 389.14 | 7.46 | No HAART | 958 | 39.6 | <20 | 1.30 |
| Interruption/Post CHER Visit | 404.7 | 414.43 | 7.94 | No HAART | 734 | 39.6 | <20 | 1.30 |
| Interruption/Post CHER Visit | 425.0 | 434.71 | 8.33 | No HAART | 918 | 38.1 | <20 | 1.30 |
| Interruption/Post CHER Visit | 430.4 | 440.14 | 8.44 | No HAART | 810 | 41.2 | <20 | 1.30 |
| Interruption/Post CHER Visit | 430.4 | 440.14 | 8.44 | No HAART | 810 | 41.2 | <20 | 1.30 |
| Interruption/Post CHER Visit | 457.4 | 467.14 | 8.95 | No HAART | 975 | 30.1 | <20 | 1.30 |
| Interruption/Post CHER Visit | 484.9 | 494.57 | 9.48 | No HAART | 892 | 38.6 | <20 | 1.30 |

**Supplementary Table 2.** Baseline CD4 counts and CD4 percentages of 227 early treated children who stopped ART in the CHER trial.

| No. | Randomisation date | CD4 count | CD4 percentage |
|---|---|---|---|
| 1 | 29Jul2005 | 1411 | 45.4 |
| 2 | 04Aug2005 | 1292 | 34.8 |
| 3 | 19Aug2005 | 2122 | 38.4 |
| 4 | 25Aug2005 | 2192 | 29.6 |
| 5 | 07Sep2005 | 2255 | 36.8 |
| 6 | 15Sep2005 | 1981 | 35.6 |
| 7 | 15Sep2005 | 2554 | 41.2 |
| 8 | 26Sep2005 | 2645 | 39.1 |
| 9 | 26Sep2005 | 3071 | 45.1 |
| 10 | 28Sep2005 | 2610 | 39.3 |
| 11 | 11Oct2005 | 2142 | 33.3 |
| 12 | 12Oct2005 | 1804 | 43.8 |
| 13 | 17Oct2005 | 2117 | 28.6 |
| 14 | 17Oct2005 | 1482 | 35.8 |
| 15 | 02Nov2005 | 1772 | 32.5 |
| 16 | 08Nov2005 | 2033 | 33 |
| 17 | 02Dec2005 | 2436 | 40.1 |
| 18 | 08Dec2005 | 3376 | 45.7 |
| 19 | 14Dec2005 | 2806 | 26.5 |
| 20 | 21Dec2005 | 3086 | 37.6 |
| 21 | 21Dec2005 | 2416 | 37.7 |
| 22 | 04Jan2006 | 2600 | 30.9 |
| 23 | 23Jan2006 | 1863 | 28 |
| 24 | 25Jan2006 | 1108 | 31.1 |
| 25 | 25Jan2006 | 2012 | 36.3 |
| 26 | 25Jan2006 | 2917 | 40.2 |
| 27 | 03Feb2006 | 1923 | 31.5 |
| 28 | 03Feb2006 | 1739 | 34.6 |
| 29 | 16Feb2006 | 2339 | 40.6 |
| 30 | 17Feb2006 | 1368 | 27.6 |
| 31 | 23Feb2006 | 2763 | 38.9 |
| 32 | 24Feb2006 | 2361 | 34 |
| 33 | 24Feb2006 | 1543 | 32.4 |
| 34 | 27Feb2006 | 2047 | 42.4 |
| 35 | 27Feb2006 | 1705 | 33 |
| 36 | 03Mar2006 | 2370 | 42.2 |
| 37 | 08Mar2006 | 3340 | 32.4 |
| 38 | 09Mar2006 | 4258 | 44.4 |
| 39 | 13Mar2006 | 1488 | 35.1 |
| 40 | 17Mar2006 | 3292 | 43.4 |

| | | | |
|---|---|---|---|
| 41 | 17Mar2006 | 2387 | 28.1 |
| 42 | 24Mar2006 | 2643 | 36.1 |
| 43 | 27Mar2006 | 3900 | 28.9 |
| 44 | 05Apr2006 | 3403 | 39.1 |
| 45 | 10Apr2006 | 2625 | 48.7 |
| 46 | 10Apr2006 | 1772 | 31.1 |
| 47 | 10Apr2006 | 1822 | 37.5 |
| 48 | 12Apr2006 | 4561 | 35.3 |
| 49 | 19Apr2006 | 2201 | 25.3 |
| 50 | 20Apr2006 | 1763 | 26.9 |
| 51 | 20Apr2006 | 3773 | 33.8 |
| 52 | 25Apr2006 | 2292 | 31.4 |
| 53 | 11May2006 | 2477 | 39.1 |
| 54 | 15May2006 | 1075 | 36.4 |
| 55 | 30May2006 | 3439 | 41.7 |
| 56 | 30May2006 | 1530 | 28.5 |
| 57 | 31May2006 | 820 | 30.2 |
| 58 | 05Jun2006 | 4368 | 36.4 |
| 59 | 06Jun2006 | 2457 | 37 |
| 60 | 07Jun2006 | 3085 | 37.6 |
| 61 | 20Jun2006 | 2012 | 42.3 |
| 62 | 22Jun2006 | 2334 | 37.8 |
| 63 | 22Jun2006 | 2210 | 59.4 |
| 64 | 26Jun2006 | 2923 | 43.8 |
| 65 | 27Jun2006 | 1817 | 34.2 |
| 66 | 03Jul2006 | 3185 | 28.7 |
| 67 | 03Jul2006 | 2948 | 26.8 |
| 68 | 04Jul2006 | 3369 | 37.4 |
| 69 | 05Jul2006 | 3729 | 38.6 |
| 70 | 06Jul2006 | 2924 | 36.3 |
| 71 | 10Jul2006 | 2722 | 42.2 |
| 72 | 12Jul2006 | 2098 | 25.2 |
| 73 | 12Jul2006 | 5347 | 52.6 |
| 74 | 19Jul2006 | 1309 | 33.1 |
| 75 | 21Jul2006 | 2378 | 38.2 |
| 76 | 21Jul2006 | 950 | 29.1 |
| 77 | 24Jul2006 | 3308 | 41.9 |
| 78 | 24Jul2006 | 3841 | 54.5 |
| 79 | 25Jul2006 | 2982 | 40.1 |
| 80 | 27Jul2006 | 1290 | 32.7 |
| 81 | 31Jul2006 | 2480 | 38.6 |
| 82 | 08Aug2006 | 2662 | 27.3 |
| 83 | 10Aug2006 | 2447 | 39.7 |
| 84 | 10Aug2006 | 1793 | 36.1 |
| 85 | 11Aug2006 | 2212 | 29.5 |

| | | | |
|---|---|---|---|
| 86 | 17Aug2006 | 1759 | 28.8 |
| 87 | 21Aug2006 | 2810 | 49.9 |
| 88 | 23Aug2006 | 2136 | 35.9 |
| 89 | 24Aug2006 | 2283 | 36.4 |
| 90 | 25Aug2006 | 2211 | 35.1 |
| 91 | 29Aug2006 | 4572 | 40.9 |
| 92 | 30Aug2006 | 4828 | 41.8 |
| 93 | 31Aug2006 | 3707 | 45.7 |
| 94 | 04Sep2006 | 1125 | 25.4 |
| 95 | 05Sep2006 | 2433 | 36 |
| 96 | 06Sep2006 | 2351 | 31.8 |
| 97 | 13Sep2006 | 2765 | 42.6 |
| 98 | 15Sep2006 | 3432 | 37.2 |
| 99 | 15Sep2006 | 2017 | 31 |
| 100 | 21Sep2006 | 1873 | 32.4 |
| 101 | 21Sep2006 | 3017 | 42.8 |
| 102 | 22Sep2006 | 1609 | 32.2 |
| 103 | 26Sep2006 | 2198 | 53 |
| 104 | 27Sep2006 | 1708 | 28.1 |
| 105 | 27Sep2006 | 5717 | 44.7 |
| 106 | 29Sep2006 | 1962 | 36.6 |
| 107 | 09Oct2006 | 1502 | 25.1 |
| 108 | 11Oct2006 | 1867 | 34.4 |
| 109 | 11Oct2006 | 3695 | 49.4 |
| 110 | 18Oct2006 | 2461 | 28.7 |
| 111 | 23Oct2006 | 1552 | 33.6 |
| 112 | 23Oct2006 | 1213 | 27.2 |
| 113 | 25Oct2006 | 3270 | 38.7 |
| 114 | 31Oct2006 | 1564 | 27.8 |
| 115 | 31Oct2006 | 3091 | 31.7 |
| 116 | 06Nov2006 | 519 | 30.6 |
| 117 | 06Nov2006 | 3346 | 62 |
| 118 | 10Nov2006 | 2200 | 29.8 |
| 119 | 10Nov2006 | 2625 | 29.47 |
| 120 | 14Nov2006 | 4339 | 48.7 |
| 121 | 20Nov2006 | 4738 | 32.9 |
| 122 | 29Nov2006 | 1853 | 35.8 |
| 123 | 29Nov2006 | 2176 | 25.6 |
| 124 | 29Nov2006 | 975 | 26.1 |
| 125 | 30Nov2006 | 2087 | 33.6 |
| 126 | 04Dec2006 | 1695 | 34.6 |
| 127 | 05Dec2006 | 2942 | 47.9 |
| 128 | 07Dec2006 | 3260 | 48.2 |
| 129 | 07Dec2006 | 4131 | 47.9 |
| 130 | 11Dec2006 | 3725 | 49.8 |

| | | | |
|---|---|---|---|
| 131 | 11Dec2006 | 3460 | 33.7 |
| 132 | 18Dec2006 | 1479 | 33.3 |
| 133 | 21Dec2006 | 2512 | 37.2 |
| 134 | 22Dec2006 | 4077 | 38.8 |
| 135 | 28Dec2006 | 1973 | 49.3 |
| 136 | 29Dec2006 | 2331 | 33.4 |
| 137 | 02Jan2007 | 2359 | 54.2 |
| 138 | 05Jan2007 | 2442 | 25.2 |
| 139 | 09Jan2007 | 1471 | 43.7 |
| 140 | 10Jan2007 | 2727 | 51.7 |
| 141 | 19Jan2007 | 2285 | 43.4 |
| 142 | 24Jan2007 | 3345 | 40.2 |
| 143 | 25Jan2007 | 1537 | 31 |
| 144 | 30Jan2007 | 1985 | 36.3 |
| 145 | 31Jan2007 | 2835 | 46.4 |
| 146 | 01Feb2007 | 2342 | 41 |
| 147 | 02Feb2007 | 2910 | 44.9 |
| 148 | 08Feb2007 | 4858 | 58.8 |
| 149 | 12Feb2007 | 2995 | 36.6 |
| 150 | 19Feb2007 | 2087 | 25.2 |
| 151 | 22Feb2007 | 3081 | 39.6 |
| 152 | 19Nov2007 | 2785 | 27.7 |
| 153 | 26Nov2007 | 4411 | 26 |
| 154 | 26Nov2007 | 2096 | 28.6 |
| 155 | 05Dec2007 | 3116 | 27.7 |
| 156 | 06Dec2007 | 1929 | 39.2 |
| 157 | 06Dec2007 | 4213 | 43.7 |
| 158 | 10Dec2007 | 1652 | 27.9 |
| 159 | 11Dec2007 | 3657 | 49.1 |
| 160 | 27Dec2007 | 3301 | 45.8 |
| 161 | 27Dec2007 | 1958 | 38.8 |
| 162 | 03Jan2008 | 3118 | 29.7 |
| 163 | 04Jan2008 | 2249 | 41.6 |
| 164 | 08Jan2008 | 4926 | 31.3 |
| 165 | 09Jan2008 | 1990 | 28.2 |
| 166 | 11Jan2008 | 3902 | 51.5 |
| 167 | 11Jan2008 | 3621 | 46.1 |
| 168 | 16Jan2008 | 2037 | 31.2 |
| 169 | 17Jan2008 | 2465 | 46.7 |
| 170 | 18Jan2008 | 3168 | 39.4 |
| 171 | 30Jan2008 | 2144 | 44 |
| 172 | 31Jan2008 | 1293 | 31.1 |
| 173 | 31Jan2008 | 2619 | 40 |
| 174 | 06Feb2008 | 2170 | 31.4 |
| 175 | 08Feb2008 | 2415 | 52.1 |

| | | | |
|---|---|---|---|
| 176 | 14Feb2008 | 1788 | 40.5 |
| 177 | 18Feb2008 | 2288 | 47.3 |
| 178 | 02Sep2005 | 6075 | 44 |
| 179 | 23Sep2005 | 2283 | 68 |
| 180 | 30Sep2005 | 1458 | 44 |
| 181 | 23Dec2005 | 1482 | 31.5 |
| 182 | 25Jan2006 | 1289 | 27.1 |
| 183 | 30Jan2006 | 3103 | 41.1 |
| 184 | 06Feb2006 | 1523 | 45 |
| 185 | 14Mar2006 | 908 | 30.5 |
| 186 | 10Apr2006 | 1923 | 32.7 |
| 187 | 10Apr2006 | 2972 | 45.4 |
| 188 | 12May2006 | 1304 | 33.1 |
| 189 | 18May2006 | 2919 | 36.7 |
| 190 | 14Jun2006 | 1504 | 29.7 |
| 191 | 28Jun2006 | 2217 | 51.1 |
| 192 | 03Jul2006 | 2357 | 37.8 |
| 193 | 05Jul2006 | 1387 | 42.5 |
| 194 | 05Jul2006 | 2236 | 36.2 |
| 195 | 07Jul2006 | 1965 | 54 |
| 196 | 15Aug2006 | 1745 | 28.8 |
| 197 | 23Aug2006 | 1498 | 38.3 |
| 198 | 04Sep2006 | 2172 | 40.4 |
| 199 | 06Sep2006 | 1626 | 36 |
| 200 | 15Sep2006 | 1237 | 41 |
| 201 | 20Sep2006 | 1235 | 29.5 |
| 202 | 21Sep2006 | 1551 | 38.5 |
| 203 | 28Sep2006 | 1794 | 45.9 |
| 204 | 03Oct2006 | 1743 | 32.2 |
| 205 | 03Oct2006 | 1453 | 31.5 |
| 206 | 04Oct2006 | 1399 | 28.1 |
| 207 | 11Oct2006 | 1441 | 34.7 |
| 208 | 26Oct2006 | 3150 | 39.4 |
| 209 | 31Oct2006 | 2420 | 55.8 |
| 210 | 09Nov2006 | 432 | 27.2 |
| 211 | 10Nov2006 | 2018 | 44 |
| 212 | 16Nov2006 | 1137 | 37.2 |
| 213 | 17Nov2006 | 1661 | 43 |
| 214 | 24Nov2006 | 1218 | 26.2 |
| 215 | 05Dec2006 | 1987 | 26.8 |
| 216 | 05Dec2006 | 3011 | 38.4 |
| 217 | 12Dec2006 | 1350 | 32.9 |
| 218 | 03Jan2007 | 2949 | 54.2 |
| 219 | 22Jan2007 | 1410 | 53.8 |
| 220 | 23Jan2007 | 918 | 36.4 |

| | | | |
|---|---|---|---|
| 221 | 31Jan2007 | 3958 | 43.5 |
| 222 | 05Feb2007 | 2750 | 44.6 |
| 223 | 12Feb2007 | 1845 | 35.6 |
| 224 | 20Feb2008 | 2496 | 35.4 |
| 225 | 26Feb2008 | 2004 | 33.2 |
| 226 | 26Feb2008 | 1997 | 31 |
| 227 | 05Mar2008 | 815 | 33.6 |

**Supplementary Table 3.** Immunophenotyping data for subsets of CD4 and CD8 T-cells (manuscript Fig. 5a-c).

**Fig. 5a**

| | CD4:CD8 ratio |
|---|---|
| **Case** | 1.87 |
| Uninfected Children | 1.45 |
| | 1.37 |
| | 1.31 |
| | 1.74 |
| | 1.59 |
| Uninfected Adults | 1.64 |
| | 2.00 |
| | 1.69 |
| | 1.55 |
| | 1.81 |
| | 0.84 |
| | 2.56 |
| | 1.62 |
| | 1.06 |
| | 0.54 |

**Fig. 5b**

| | Memory T-cell subsets (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **CD4+ T-cells** | | | | | | **CD8+ T-cells** | | | | | |
| | Naïve | SCM | CM | TM | EM | TE | Naïve | SCM | CM | TM | EM | TE |
| **Case** | 54.9 | 0.587 | 21.5 | 6.33 | 6.81 | 0.07 | 59.2 | 0.664 | 5.12 | 4.13 | 18.3 | 5.32 |
| Uninfected Children | 61.1 | 1.28 | 15.8 | 10.3 | 4.2 | 0.06 | 42.2 | 0.444 | 3.67 | 11.8 | 13.9 | 10.2 |
| | 46.5 | 2.13 | 24.5 | 8.07 | 6.99 | 1.95 | 32.1 | 0.873 | 2.94 | 6.83 | 19 | 23.6 |
| | 64 | 0.688 | 19.5 | 6.27 | 3.1 | 0.30 | 43.8 | 0.721 | 3.11 | 2.1 | 4.8 | 22.8 |
| | 53.6 | 2.68 | 21.3 | 8.75 | 4.84 | 0.03 | 58.5 | 2.43 | 3.74 | 6.28 | 9.8 | 7.31 |
| | 64.2 | 1.87 | 16.4 | 5.49 | 3.86 | 0.24 | 51.8 | 1.04 | 3.07 | 6.97 | 12.6 | 11.7 |
| Uninfected Adults | 41.8 | 2.87 | 28.2 | 5.6 | 6.4 | 4.46 | 49.7 | 1.03 | 2.88 | 5.85 | 11.9 | 12.6 |
| | 42 | 2.3 | 32.6 | 5.83 | 5.33 | 0.07 | 50.9 | 0.99 | 8.85 | 11.9 | 18.4 | 2.21 |
| | 41.8 | 2.39 | 28.2 | 10.5 | 8.82 | 0.21 | 40 | 0.419 | 5.88 | 20.1 | 16.7 | 5.6 |
| | 27.1 | 2.98 | 31.9 | 9.9 | 13.9 | 1.81 | 17 | 0.911 | 5.71 | 10.1 | 22.4 | 24.9 |
| | 45 | 5.12 | 28.5 | 6.69 | 4.29 | 0.32 | 32.9 | 1.15 | 5.9 | 9.62 | 14.9 | 21.5 |
| | 27.2 | 1.8 | 32.5 | 6.93 | 11.3 | 4.01 | 14.8 | 0.318 | 5.91 | 15.9 | 29.9 | 18.9 |
| | 30.9 | 2.19 | 47.3 | 2.95 | 3.28 | 0.05 | 50.1 | 1.08 | 8.72 | 6.44 | 14.5 | 5.73 |
| | 24.7 | 1.74 | 42.4 | 10 | 8.21 | 0.17 | 6.76 | 0.431 | 5.32 | 8.64 | 18.9 | 11.5 |
| | 44 | 2.61 | 21.9 | 5.97 | 9.91 | 5.75 | 49.6 | 1.5 | 3.05 | 3.15 | 6.27 | 24.9 |
| | 9.44 | 6.1 | 53 | 7.43 | 8.45 | 0.58 | 10.6 | 1.78 | 7.64 | 6.41 | 9.47 | 39 |

**Fig. 5c**

**CCR5, activation and immune-checkpoint markers**

| | **CD4+ T-cells** | | | | | **CD8+ T-cells** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CCR5 density | CCR5% | HLA-DR | TIGIT | PD-1 | CCR5 density | CCR5% | HLA-DR | TIGIT | PD-1 |
| **Case** | 139.42 | 11.2 | 2.18 | 16.3 | 17.9 | 163.43 | 18.3 | 5.26 | 22.6 | 10.5 |
| Uninfected Children | 198.15 | 10.2 | 2.86 | 11 | 7.89 | 242.9 | 21.9 | 5.04 | 22.5 | 2.92 |
| | 148.22 | 14.1 | 7.93 | 21 | 6.69 | 221.05 | 17.8 | 10.6 | 31.2 | 3.31 |
| | 266.84 | 10.2 | 2.45 | 13.9 | 6.57 | 287.16 | 9.86 | 2.62 | 22.9 | 2.94 |
| | 186.64 | 12.9 | 3.6 | 15.5 | 7.61 | 250.56 | 17.9 | 5.03 | 17.4 | 3.47 |
| | 124.23 | 6.13 | 2.01 | 12.7 | 4.35 | 158.09 | 14.2 | 5.16 | 20 | 1.68 |
| Uninfected Adults | 164.16 | 4.69 | 2.45 | 12.7 | 7.17 | 142.02 | 21.4 | 4.51 | 25.6 | 6.7 |
| | 212.22 | 16 | 0.97 | 15.6 | 7.4 | 367.92 | 33.7 | 1.91 | 29.4 | 10.7 |
| | 202.22 | 12.2 | 1.67 | 21.4 | 17.6 | 231.41 | 34.7 | 2.58 | 41.2 | 6.32 |
| | 147.62 | 10.3 | 3.16 | 19.8 | 6.18 | 171.47 | 44.2 | 3.87 | 46.4 | 6.76 |
| | 166.08 | 10.5 | 2.36 | 21.8 | 9.56 | 245.83 | 64.5 | *53.3 | 46 | 6.67 |
| | 190.22 | 28.4 | 4.04 | 22.7 | 4.57 | 416.1 | 38.1 | 10.1 | 42.6 | 8.05 |
| | 176.15 | 8.48 | 2.59 | 25.2 | 10.8 | 211.03 | 29.6 | 10.2 | 39.4 | 21.8 |
| | 159.89 | 19.4 | 0.91 | 16.6 | 3.97 | 282.03 | 54.3 | 2.58 | 62.6 | 16.3 |
| | 170.56 | 20.4 | 2.55 | 22.7 | 5.93 | 201.54 | 36.7 | 8.97 | 39.2 | 11.9 |
| | 193.78 | 14 | 0.61 | 26 | 20.5 | 212.37 | 39.7 | 1.5 | 59.3 | 16.7 |

*Data point (extreme outlier) excluded from Fig. 5c graph – no significant difference between children and adults with or without this data point (two-tailed Mann-Whitney U-test, P>0.05).

**Supplementary Table 4.** Source of the HIV-1 antigens coupled to carboxylated magnetic beads (Bio-Rad) and the bead regions selected.

| HIV-antigen | HIV-1 subtype | Catalogue number | Source | Fluorescence region |
|---|---|---|---|---|
| gp120 | C | | Virology, NICD, Prof L Morris | 026 |
| gp41 | unknown | PR-1204 | Jena Bioscience, Germany | 034 |
| Vpu-43$^{GST}$ | unknown | PR-380 | Jena Bioscience, Germany | 043 |
| RT wild type (H66/H51) | unknown | 3555 | NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, from Dr Stuart Le Grice and Dr Jennifer T Miller[2] | 052 |
| HIV-1 Nef | unknown | 11478 | NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH | 055 |
| HIV-$1_{IIIB}$ pr55 Gag | HIV-$1_{IIIB}$ strain | 3276 | NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH | 062 |
| V1V2 scaffold | C (CAP257) | | Virology, NICD, Prof L Morris | 063 |
| Vpu 9 | C (STMVDMGHLR LLDVNDL) | | NMI Natural and Medical Sciences Institute, University Tuebingen, Germany | 064 |
| HIV-$1_{IIIB}$ Tat | HIV-$1_{IIIB}$ strain | 2222 | NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH[3] | 065 |
| HIV immunoglobulin (Positive control) | Asymptomatic HIV antibody positive donors | 3957 | NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, from NABI and National Heart Lung and Blood Institute (Dr Luiz Barbosa) | |

**Supplementary Table 5.** PCR primer sequences used for the real-time AS-PCR *KIR* genotyping assay.

| Well | *KIR* gene target | Primer sequence (5'-3') | *KIR* alleles not detected |
|---|---|---|---|
| 1 | [a]2DL1 f1 | GTTGGTCAGATGTCATGTTTGAA | *013N |
| | [a]2DL1 r1 | GGTCCCTGCCAGGTCTTGCG | |
| 2 | [a]2DL1 f2 | TGGACCAAGAGTCTGCAGGA | *005 |
| | [a]2DL1 r2 | TGTTGTCTCCCTAGAAGACG | *0040102 |
| 3 | [a]2DL2 f1 | CTGGCCCACCCAGGTCG | *004;*00601;*00602 |
| | [a]2DL2 r1 | GGACCGATGGAGAAGTTGGCT | *00303;*004 |
| 4 | [b]2DL2 f2 | AAACCTTCTCTCTCAGCCCA | |
| | [b]2DL2 r2 | GCCCTGCAGAGAACCTACA | *009 |
| 5 | [b] 2DL3 f1 | AGACCCTCAGGAGGTGA | |
| | [b] 2DL3 r1 | CAGGAGACAACTTTGGATCA | *010;*017 |
| 6 | [d]2DL3 f2 | GGCCCAGAAGTGCCCTCT | |
| | [d]2DL3 r2 | ATTGGAGCTGGCAACCCA | |
| 7 | [a] 2DL4 f1 | CAGGACAAGCCCTTCTGC | |
| | [a]2DL4 r1 | CTGGGTGCCGACCACT | |
| 8 | [a]2DL4 f2 | ACCTTCGCTTACAGCCCG | |
| | [a]2DL4 r2 | CCTCACCTGTGACAGAAACAG | |
| 9 | [a]2DL5 f1 | GCGCTGTGGTGCCTCG | |
| | [a]2DL5 r1 | GACCACTCAATGGGGGAGC | |
| 10 | [a]2DL5 f2 | TGCAGCTCCAGGAGCTCA | |
| | [a]2DL5 r2 | GGGTCTGACCACTCATAGGGT | |
| 11 | [b]2DS1 f1a | TCTCCATCAGTCGCATGAG | |
| | [b]2DS1 f1b | TCTCCATCAGTCGCATGAA | |
| | [b]2DS1 r1 | GGTCACTGGGAGCTGAC | |
| 12 | [d]2DS1 f2 | CCGCTCTTGAGCGAGCAA | |
| | [d]2DS1 r2 | CAACTCCACCTCCAGGCCTATATA | |
| 13 | [a]2DS2 f1 | TTCTGCACAGAGAGGGGAAGTA | |
| | [a]2DS2 r1 | GGGTCACTGGGAGCTGACAA | |
| 14 | [a] 2DS2 f2 | CGGGCCCCACGGTTT | *00104 |
| | [a] 2DS2 r2 | GGTCACTCGAGTTTGACCACTCA | |
| 15 | [c]2DS3 f1 | AAACCTTCTCTCTCAGCCCA | |
| | [c]2DS3 r1 | GCATCTGTAGGTTCCTCCT | |
| 16 | [a] 2DS3 f2 | CTATGACATGTACCATCTATCCAC | |

| | | | |
|---|---|---|---|
| | [a] 2DS3 r2 | AAGCAGTGGGTCACTTGAC | |
| 17 | [a] 2DS4 f1 | CTGGCCCTCCCAGGTCA | |
| | [a] 2DS4 r1 | TCTGTAGGTTCCTGCAAGGACAG | |
| 18 | [a] 2DS4 f2 | GTTCAGGCAGGAGAGAAT | |
| | [a] 2DS4 r2 | GTTTGACCACTCGTAGGGAGC | |
| 19 | [a]2DS5 f1 | TGATGGGGTCTCCAAGGG | |
| | [a]2DS5 r1 | TCCAGAGGGTCACTGGGC | *003 |
| 20 | [a]2DS5 f2 | ACAGAGAGGGGACGTTTAACC | |
| | [a]2DS5 r2 | ATGTCCAGAGGGTCACTGGG | |
| 21 | [a]2DP1 f1 | GTCTGCCTGGCCCAGCT | |
| | [a]2DP1 r1 | GTGTGAACCCCGACATCTGTAC | |
| 22 | [a]2DP1 f2 | CCATCGGTCCCATGATGG | |
| | [a]2DP1 r2 | CACTGGGAGCTGACAACTGATG | |
| 23 | [a]3DL1 f1 | CGCTGTGGTGCCTCGA | *009;*042;*057 |
| | [a]3DL1 r1 | GGTGTGAACCCCGACATG | |
| 24 | [b]3DL1 f1a | CCATCGGTCCCATGATGCT | *054 |
| | [b]3DL1 f1b | CCATTGGTCCCATGATGCT | *054 |
| | [b]3DL1 f1c | TCCATCGGTCCCATGATGTT | *054 |
| | [b]3DL1 r2 | CCACGATGTCCAGGGGA | |
| 25 | [a]3DL2 f1 | CAAACCCTTCCTGTCTGCCC | *01301;*01302;*014;*024;*025;<br>*037;*040;*046;*052;*054 |
| | [a]3DL2 r1 | GTGCCGACCACCCAGTGA | |
| 26 | [a]3DL2 f2 | CCCATGAACGTAGGCTCCG | |
| | [a]3DL2 r2 | CACACGCAGGGCAGGG | *018 |
| 27 | [a]3DL3 f1 | GTCAGGACAAGCCCTTCCTC | |
| | [a]3DL3 r1 | GAGTGTGGGTGTGAACTGCA | |
| 28 | [a]3DL3 f2 | TTCTGCACAGAGAGGGGATCA | |
| | [a]3DL3 r2 | GAGCCGACAACTCATAGGGTA | |
| 29 | [a]3DS1 f1 | AGCCTGCAGGGAACAGAAG | |
| | [a]3DS1 r1 | GCCTGACTGTGGTGCTCG | |
| 30 | [b]3DS1f 2a | CATCGGTTCCATGATGCG | |
| | [b]3DS1f 2b | CATCAGTTCCATGATGCG | |
| | [b]3DS1 r2 | CCACGATGTCCAGGGGA | |
| 31 | [b]3DP1 f1 | GTACGTCACCCTCCCATGATGTA | *00902;*004 |
| | [d]3DP1 r1 | GCACTCCCTCCCTCTATTCCT | |
| 32 | [d]3DP1 f2 | CATCCTCCTCTCTAAGGTGGC | |
| | [b]3DP1 r2 | GAAAACGGTGTTTCGGAATAC | |

| | | | |
|---|---|---|---|
| All | [c]GALC f | TTACCCAGAGCCCTATCGTTCT | |
| | [c]GALC r | GTCTGCCCATCACCACCTATT | |

GALC, galactosylceramidase; KIR, killer-cell immunoglobulin-like receptor; PCR, polymerase chain reaction; AS-PCR, allele-specific PCR; Primers taken from [a]Martin and Carrington[4], [b]Vilches *et al.*[5], [c]Alves *et al.*[6], and [d]Gentle *et al*.[7]

**Supplementary Table 6.** List of PCR primers and probe sequences used for real-time quantitative AS-PCR copy number determination of KIR3DL1/S1 and KIR2DS4f/v.

| Well | Primer name | Primer sequence (5'-3') |
|---|---|---|
| 1 | *KIR3DL1* f1[a] | GCCTCGTTGGACAGATCCA[T] |
| | *KIR3DL1* r1[a] | TAGGTCCCTGCAAGGGCA[A] |
| | *KIR3DL1/S1* probe[a] | VIC-GGGTCTCCAAGGCCAATTTCTCCAT-MGB |
| 2 | *KIR3DS1* f1[a] | CTCGTTGGACAGATCCATG[A] |
| | *KIR3DS1* r1[a] | GTCCCTGCAAGGGCA[C] |
| | *KIR3DL1/S1* probe[a] | VIC-GGGTCTCCAAGGCCAATTTCTCCAT-MGB |
| 3 | *KIR2DS4f* f1[b] | CCGGAGCTCCTATGACATG |
| | *KIR2DS4f/v* r1[b] | TGACGGAAACAAGCAGTGG[A] |
| | *KIR2DS4f/v* probe[b] | VIC-AACATTCCAGGCCGACTTTCCTCTG-MGB |
| 4 | *KIR2DS4v* f1[b] | CCTTGTCCTGCAGCTCCAT |
| | *KIR2DS4f/v* r1[b] | TGACGGAAACAAGCAGTGG[A] |
| | *KIR2DS4f/v* probe[2] | VIC-AACATTCCAGGCCGACTTTCCTCTG-MGB |
| All | *BGB* f1[c] | TCGCTTTCTTGCTGTCCAATTTCTA |
| | *BGB* r1[c] | ATGCTCAAGGCCCTTCATAATATCC |
| | *BGB* probe[c] | FAM-CCTAAGTCCAACTACTAAACTG-MGB |

VIC and FAM, probe reporter fluorescent dyes; MGB, minor groove binding protein (non-fluorescent quencher); AS-PCR, allele-specific PCR; *BGB*, human beta-globin, [ ] denotes 3' locked nucleic acid (LNA) base modification. [a]Primers/probes taken from Pelak *et al*.[8], [b]Jiang *et al.*[9], and [c]Shostakovich-Koretskaya *et al*.[10]

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