Figure S1.
Figure S2.
Figure S2.
a) Paired-end analysis of two interacting fragments

b) Paired-end analysis of simultaneously interacting fragments

Figure S3.
Figure S4.
Figure S5.
Legends to Supplementary Figures

Figure S1. The importance of a re-ligation step to improve the efficiency of the 4C assay. The terminal 220kb of chromosome 16 (16p13.3) is shown as in the main text. Below this, represented by red boxes are the MCS-R elements of the α-globin genes. Two tracks showing data from amplified 4C libraries using primers within the α-globin promoters (marked with red asterisks). The tracks compare data with a single or double ligation protocol. The microarray platform is a custom Affymetrix genomic tiled array covering the terminal 220 Kb of chromosome 16 described previously.(1).

Figure S2. Analysis of CTCF interactions in the α-globin locus using 4C on tiled genomic microarrays: a control for 4C interactions in lymphoblastoid cells. (a) The terminal region of chromosome 16 (16p13.3) is displayed as in the main text. (b) Two biological replicates of 4C amplifications from the 4C libraries derived from B-lymphocytes (shown in figure 3 in the main text). The bait is a CTCF bound region upstream of the Axin1 gene promoter (marked with a red asterisk). (c) Released datasets for the analysis of B-lymphocytes (Gm12878) DNaseI hypersensitive sites (Open Chromatin, Encode) and of T-lymphocytes (CD4)(2) CTCF occupancy and H3K4me3. Examples are highlighted of the coincidence of the interactions in b, with DNase1 sites, other CTCF bound regions and active promoter marks. Interactions with other CTCF bound regions are indicated by dashed black lines. Interactions with H3K4Me3 enriched promoters are indicated by dashed red lines.
Figure S3. Methods for analysing HTS sequencing data. (a) Illustrates paired end analysis of 4C material involving two interacting fragments. The circular 4C template is shown as a black (bait) and red (captured) circle. Dpn II sites are marked D. The primers used for the inverse PCR are represented as arrows. The amplified inverse PCR product is shown below this as a linear molecule. The paired sequence reads P1 and P2 are generated from sonicated fragments of the PCR product and are distributed across its length. As each set of P1 and P2 reads come from the same fragment of DNA, when one paired-end read maps to a black fragment and its matching read maps to a red fragment this represents a true ligation event between the two fragments. As the material is sonicated then the junction analysis is no longer restricted to the region adjacent to the Dpn II site. (b) Analysis of circular inverse PCR template involving three interacting fragments (black bait fragment, red and green captured fragments). Paired-end analysis is performed as described in (a). However, now, it is also possible to detect an interaction between the interacting red and green fragments. This analysis can be extended to map one interaction using a read, which lies over a junction (P2 read in green and black shown below the line) and its matching read which lies over a third interacting fragment (red P1 below the line). This analysis has the potential to map three interactions on the same DNA fragment.

Figure S4. HTS analysis of single molecular interactions in the α-globin active chromatin hub. (a) A detailed view of the α-globin locus between coordinates chr16:78000-139000. Below this is a track of the HTS whole genome mapping of CTCF occupancy in CD4+ cells(2). Below this (represented by red boxes) are the multi-species conserved sequences associated with the α-globin regulatory elements (MCS-R1–4). Dnase1 hypersensitive sites are shown as black diamonds and those associated with CTCF bound regions are labelled. (b)
Interactions between the MCS elements and CTCF bound regions co-amplified from the α-globin promoter, revealed by paired-end HTS analysis. Interactions with MCS-R2, co-amplified from the α-globin promoter, are highlighted in red. The region covering the Dpn II fragments, which contain the MCS-R2 element and the two flanking fragments, which share a common restriction site with this fragment, is shown as a grey bar. (c) Two tracks of CTCF occupancy across the α-globin locus taken from previously released data of chromatin immunoprecipitation using a CTCF antibody (UCSC genome browser HG18 Broad Institute Histone Modification track) in the erythroid-like cell line K562 and B-lymphocyte cell line GM12878 (Data from Open Chromatin, Encode project); the maximum scale is set to 20 fold to highlight lower enrichments. Although K562 (an erythroid cell line) and primary erythroid cells are not strictly equivalent, the peaks observed in K562 were reproduced in preliminary experiments using primary erythroid cultures (unpublished).

**Figure S5. Analysis of potential intrachromosomal interactions between the α-globin and β-globin loci.** (a) The terminal 500kb region of chromosome 16 (16p13.3 as described in Figure 2a) corresponding to the α-globin locus and the β-globin locus (chr11:50000-550000 as described in Figure 3a). (b) Inverse PCR amplification from the α-globin promoters (indicated by red asterisks) of 4C libraries derived from two biological replicates of primary erythroid cells as shown in Figure 2b. Signal from the same microarray covering the α-globin locus is shown on the left and the β-globin locus is shown on the right. The scale on the β-globin locus has been set to the same scale as the α-globin locus. (c) Inverse PCR amplification from the β-globin HBB gene promoter (indicated by a red asterisk) of 4C libraries derived from two biological replicates of primary erythroid
cells as shown in Figure 3b. Signal from the same microarray covering the α-globin locus is shown on the left and the β-globin locus is shown on the right. The scale on the α-globin locus has been set to the same scale as the β-globin locus.
