duplication, WC – whole chromosome. Events with incomplete understanding are labeled '?'.

Figure 4: Chromothripsis alters the copy number landscape of chromosome 21 in a non-random fashion.

Chromosome arm-level (**A**) and zoomed-in view (**B**) of chromosome 21, showing gene expression, copy number (CN) distribution, chromothripsis effect and distribution of rearrangement breakpoints. In the gene expression panels, positive strand genes are shown in blue and negative strand genes are shown in red. **C:** Correlation between average rate of deletion in the Beroukhim *et al.*[19] dataset and chromothripsis effect for chromosome 21. IQR – inter-quartile range.

LEGENDS FOR EXTENDED DATA ITEMS

Extended Data Table 1: Application of published criteria for chromothripsis[14] to the chromosome 21 amplifications analysed in this manuscript.

Extended Data Figure 1: FISH studies of patient PD10009a indicating the role of the centromeres in the formation of the iAMP21 chromosome from rob(15;21)c patients.

a. A representative normal metaphase from a non-leukemic cell hybridized with centromere specific probes for chromosomes 15 (CEP15, Cytocell) labelled green and chromosomes 13 and 21 (this probe cross hybridizes to both chromosomes 13 and 21, CEP13/21, Cytocell) labelled red. The chromosomes are counterstained with DAPI (blue). The discrete green signal indicates one

copy of the normal chromosome 15, the two discrete red signals at the bottom of the cell indicate the two normal copies of chromosomes 13 and the discrete red signal at the top indicates the normal chromosome 21. The closely apposed red green signals at the bottom of the cell are hybridized to the rob(15;21)c confirming that this chromosome is dicentric with intact copies of both centromeres. In order to maintain stability and normal segregation at mitosis, one centromere of a dicentric chromosome needs to be inactive. In Robertsonian translocations, chromosome 15 is most frequently inactivated. This is often depicted by decondensation of the chromatin, in contrast to tightly condensed chromatin of an active centromere. In some cells the chromosome 21 centromere of the rob(15;21)c appeared smaller (condensed) than the chromosome 15 centromere (decondensed).

b. A representative normal metaphase indicating the chromosome 15 centromere (CEP 15, green) and a probe set designed to cover the common region of amplification of chromosome 21 (CRA); probes RP11-777J19, RP11-383L18 and RP11-773I18 (as previously described) were hybridized together and labelled red. The discrete green signal indicates the centromere on the normal chromosome 15, the discrete red signal indicates the CRA on the normal chromosome 21, while the red and green signals close together show the centromere of chromosome 15 and the CRA on the rob(15;21)c.

c and e. The same representative abnormal metaphase hybridized with CEP15 (green) and CEP13/21 (red) as above. The discrete green signal indicates the intact chromosome 15 centromere on the normal chromosome 15. The three

discrete red signals show the centromeres on the two normal chromosomes 13 located either side of the red signal on the normal chromosome 21. The iAMP21 chromosome, which we know to be composed of both chromosomes 15 and 21, as patient PD7170a (Figure 2), and to be in the formation of a ring chromosome has one red signal indicating the presence of an intact chromosome 21 centromere, but absence of the green signal indicating loss of the chromosome 15 centromere, which is present in the normal cells as shown in A. This loss of chromosome 15 centromere from the der(15;21) is also confirmed by sequencing (Figure 3B). In E. the image shown in C. is inverted to confirm the origin of the chromosomes and indicate the ring formation of the iAMP21 chromosome (arrow).

d and f. The same representative abnormal metaphase hybridized with CEP15 (green) and the probe set specific for the CRA (red) as above. The discrete green signal at the bottom indicates the intact chromosome 15 centromere on the normal chromosome 15. The discrete red signal (bottom left) shows one copy of the CRA on the normal chromosome 21. The iAMP21 chromosome has multiple red signals indicating multiple copies of the CRA interspersed throughout this abnormal ring chromosome. In F the image shown in D is inverted to confirm the origin of the chromosomes and indicate the ring formation of the iAMP21 chromosome (arrow).

Extended Data Figure 2: Rearrangement orientations are distributed equally on chromosome 21 in iAMP21 patients.

Comparison of rearrangement orientations for rearrangements on chromosome 21 (left-most 4 bars for each panel) against the rest of the genome (right-most 4 for each panel) for the 9 patients sequenced. These show that the distribution was not statistically different from uniform for the chromosome 21 rearrangements (p values under x axis legend). For rearrangements in the rest of the genome, deletion-type rearrangements (TH, coloured red) predominated. Multinomial distribution statistical significances (raw *p*-values, rounded to 4 decimals) for the null hypothesis of equal distribution between all orientation types are shown in the x-axis labels.

Extended Data Figure 3: Rearrangement metrics used to describe rearranged sections of a genome.

Illustration of the process of compiling rearrangement metrics in a hypothetically rearranged chromosome (**a**) and summarization of these values into the rearrangement metrics representation used in this study (**b**). CN- copy number

Extended Data Figure 4: Rearrangement metrics of 50 simulated chromosomes under two alternative sequences of two rearrangement events, two BFB cycles and chromothripsis.

In copy number step size distribution plot, every simulated instance is represented by a point at each of the 5 copy number change size value, showing the frequency of CN steps with each CN step size. In copy number jump size distribution, rearrangements from all 50 simulated chromosomes are aggregated. In copy number trajectory, every connected line represents a single simulated chromosome.

Extended Data Figure 5. Comparison of rearrangement metrics of two alternative sequences of rearrangements that generate a chromothripsis-like CN pattern involving three CN states.

In copy number step size distribution plot, every simulated instance is represented by a point at each of the five CN change size value, showing the frequency of CN steps with each CN step size. In copy number jump size distribution, rearrangements from all 50 simulated chromosomes are aggregated. In copy number trajectory, every connected line represents a single simulated chromosome.

Extended Data Figure 6. Copy number and rearrangement pattern in the iAMP21 chromosome of patient PD9022a.

a: Copy number and rearrangement pattern of PD9022a chromosome 21. D – deletion type rearrangement link, TD – tandem duplication type, TT – tail to tail rearrangement, HH – head to head rearrangement.

b: the deletion type (tail-to-head) rearrangement resolved by five unmapped split reads whose mates mapped to the vicinity of the associated copy number breakpoint. The mapped mates and unmapped split reads are joined together by dashed lines. The 5 split reads are aligned to the two ends of the rearrangement.

Extended Data Figure 7: Fold-back-like rearrangements of patient PD7170a.

Three fold-back-like rearrangement of the der(15;21) chromosome of patient PD7170a are shown (arrows). In **a**, the rearrangement is tail-to-tail type. In **b** and **c**, the rearrangement is head-to-head type.

Extended Data Figure 8. Formation of fold-back-like rearrangements.

If two copies of the same chromosome are rearranged in the same event, fold-back-like rearrangements may form (left). If two copies of the same chromosome undergo separate rearrangements, fold-back-like rearrangements cannot form.

Extended Data Figure 9: Chromothripsis effect based on the four sequenced rob(15;21)c iAMP21 ALLs patients on chromosome 15.

REFERENCES

1 Stratton, M. R., Campbell, P. J. & Futreal, P. A. The cancer genome. *Nature* **458**, 719-724 (2009).

2 Rand, V. *et al.* Genomic characterization implicates iAMP21 as a likely primary genetic event in childhood B-cell precursor acute lymphoblastic leukemia. *Blood* **117**, 6848-6855 (2011).

3 Strefford, J. C. *et al.* Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. *Proc Natl Acad Sci U S A* **103**, 8167-8172 (2006).

4 Stiller, C. A., Kroll, M. E., Boyle, P. J. & Feng, Z. Population mixing, socioeconomic status and incidence of childhood acute lymphoblastic leukaemia in England and Wales: analysis by census ward. *Br J Cancer* **98**, 1006-1011 (2008).

5 Moorman, A. V. *et al.* Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). *Blood* **109**, 2327-2330 (2007).

6 Moorman, A. V. *et al.* Risk-directed treatment intensification significantly reduces the risk of relapse among children and adolescents with acute lymphoblastic leukemia and intrachromosomal amplification of chromosome 21: a comparison of the MRC ALL97/99 and UKALL2003 trials. *J Clin Oncol* **31**, 3389-3396 (2013).

7 Heerema, N. A. *et al.* Intrachromosomal amplification of chromosome 21 is associated with inferior outcomes in children with acute lymphoblastic leukemia treated in contemporary standard-risk children's oncology group studies: a report from the children's oncology group. *J Clin Oncol* **31**, 3397-3402 (2013).

8 Hamerton, J. L., Canning, N., Ray, M. & Smith, S. A cytogenetic survey of 14,069 newborn infants. I. Incidence of chromosome abnormalities. *Clin Genet* **8**, 223-243 (1975).

9 Jacobs, P. A., Browne, C., Gregson, N., Joyce, C. & White, H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. *J Med Genet* **29**, 103-108 (1992).