Patient samples

#	Diagnosis	Lab #	Age	Gender	WBC Count	NCI Risk	Disease Stage	Karyotype	Comments
1	ALL (B), Pediatric	10-212	1 1/2 yrs	M	2.1	SR	New Diagnosis	54,XY,+X,+4,+6,+10,+14,+17,+21,+21[15]/46,XY[5]	triple trisomy
2	ALL (B), Pediatric	11-064	8 yrs	F	20.2	SR	New Diagnosis	45,XX,del(6)(q?13),i(9)(q10),-13,der(19)t(1;19)(q2?1;p13)[15]/46,XX[5]	*TCF3/PBX1*
3	ALL (B), Pediatric	11-220	17 mon	F	14	SR	New Diagnosis	55,XX,+X,+4,+6,+10,del(10)(q2?4),+14,+17,+18,+21,+21[18]/46,XX[2]	triple trisomy
4	ALL (B), Pediatric	10-770	12 yrs	F	70	HR	New Diagnosis	46,XX[36]	
5	ALL (B), Pediatric	10-771	7 12 yrs	M	65	HR	New Diagnosis	46,XY,del(6)(q13q21),t(12;15)(p13;q15)[13]/46,XY[7], including t(12;21)	*ETV6/RUNX1*
6	ALL (B), Pediatric	10-820	13 1/2 yrs	F	3.5	HR	New Diagnosis	46,XX,t(9;15)(q13;p1?1.2)[18]/46,XX[2]	
7	ALL (B), Pediatric	11-132	15 yrs	F	70.1	HR	New Diagnosis	46,XX,t(12;17)(p13;q12)[16]/46,XX[4]	
8	ALL (B), Pediatric	11-015	4 yrs	M	>50	HR	New Diagnosis	no available data	
9	ALL (B), Pediatric	11-253	9 1/2 yrs	F	190	HR	New Diagnosis	45,XY,-20[18]/46,XY[2], including (14q32)/CRLF2 (Yp11.3)	(14q32)/*IGH@*/*CRLF2* (Yp11.3)
10	ALL (B), Pediatric	10-822	11 1/2 yrs	M	19.7	N/A	Relapse	45,Y,add(X)(p22.1),add(1)(p3?4),del(6)(q1?1.2q2?2),-9,add(12)(q2?2),t(17;19)(q22;p13.3)[1]/46,XY[24]	*E2A/HLF*
11	ALL (B), Pediatric	10-838	18 yrs	F	145.9	N/A	Relapse	46,XX[26]	Normal
12	ALL (B), Pediatric	11-118	5 yrs	F	20.1	N/A	Relapse	46,XY[20] including t(12;21)	*ETV6/RUNX1*
13	ALL (B), Pediatric	11-221	6 yrs	M	73	N/A	Secondary malignancy	46,XY,t(11;19)(q23;p13.3)[20]	11q23, treatment related secondary malignancy
14	ALL (B), Pediatric	10-668	6yrs	M	278	VHR	New Diagnosis	46,XY,del(9)(p13),t(9;22)(q34;q11.2)[12]/46,XY,del(9)(p13),der(9)t(9;22)(q34;q11.2),ider(22)(q10)t(9;22)(q34;q11.2)[4]/46,XY[7]	*BCR/ABL*
15	ALL (T), Pediatric	10-799	4 yrs	F	35	HR	New Diagnosis	46,XX,t(5;14)(q35;q11.2)[14]/46,XX[6]	
16	ALL (T), Pediatric	10-828							
17	AML M2, Pediatric	10-356	12 yr	M		N/A	New Diagnosis	46,XY,t(6;9)(p23;q34),del(15)(q11.2q15)[2]/45,sl,-Y[cp4]/46,sdl1,+4[5]/47,sdl2,+13[5]/46~47,sdl2,+mar1[cp3]/48,sdl3,+mar1[1]	*FLT3-ITD*

Leukemic cell lines

#	Diagnosis	Name	Age	Gender	Karyotype	Comments
1	ALL (B), Pediatric	REH	15 yrs	F	47,X,-X,del(3)(p12.3–p22.3),t(4;12)inv(12)(p13q23),t(5;12;16;21)(q31;p13;q24.3;q22),del(9)(p21.3),þder(16)(16;21)(q24.3;q22),t(18;21)(q11.2;p11.2). ish del(9)(p21.3).(q11.2;p11.2). ish del(9)(p21.3).(RP11615P15-RP11467K20-)	*ETV6/RUNX1*
2	ALL (B), Pediatric	RCH-ACV	8 yrs	F	47,XX,t(1;19)(q23;p13),del(6)(q14.1 q15),+8	*TCF3/PBX1*
3	ALL (B), Pediatric	MHH-CALL2	15 yrs	F	52,XX,+8,+10,+18,+18,+21,+21 [Alternative: 51,XX,+X,+18,+der(18)t(15;18)(q13.1;q22.1),+21,+21]	

Lymphoblast cell lines

#	Type	Name	Age	Gender	Karyotype
1	B-cell	C0202	35 yrs	M	46,XY
2	B-cell	GM06990	41 yrs	F	46,XX
3	B-cell	GM06999	15 yrs	F	46,XX
4	B-cell	NC-NC	27 yrs	F	45/46 XX/X, -X
5	T-cell	TC			

Figure S1. Properties of cell lines and patient samples analyzed. For patient samples obtained through COG, clinical features and NCI risk stratification are indicated, with NCI categories representing VHR: very high risk; HR: high risk; SR: standard risk. Properties listed were determined at the time of sample collection.

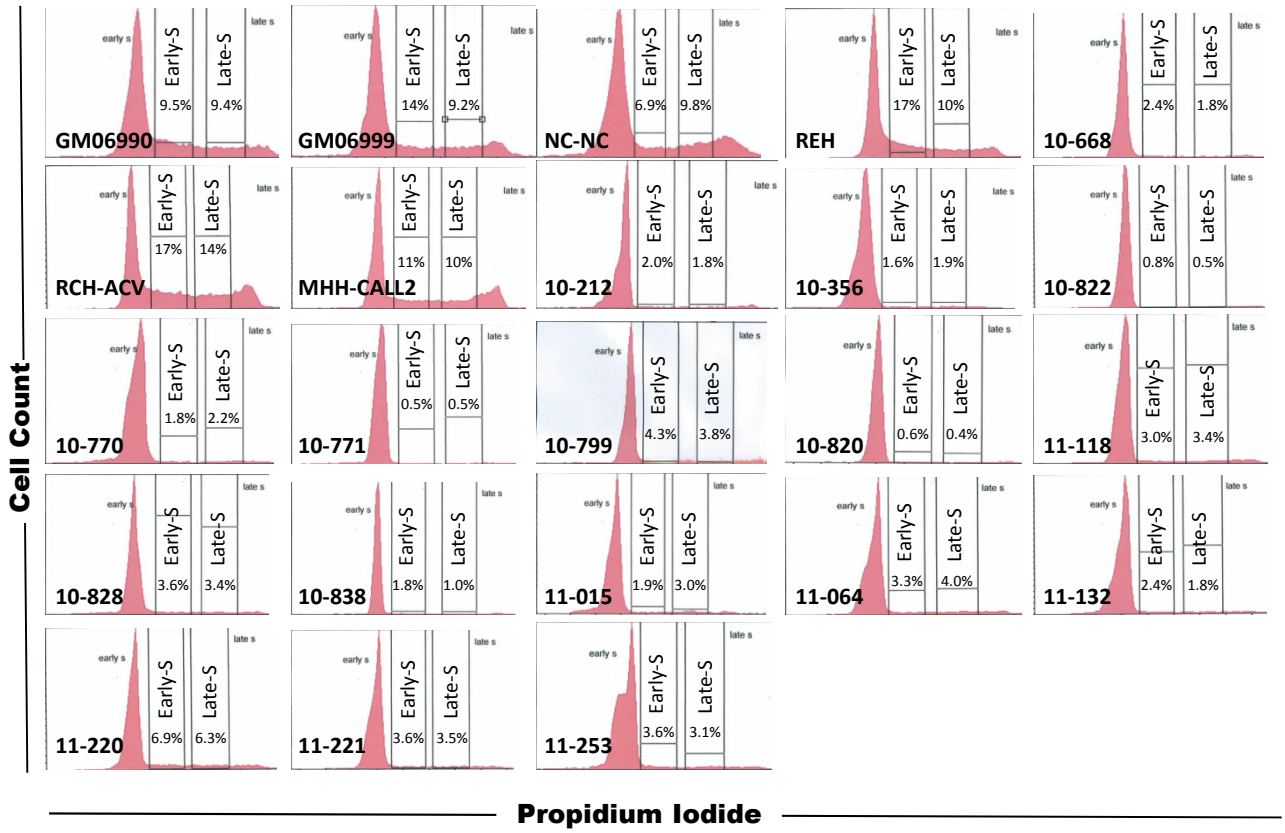

Figure S2. FACs profiles of samples analyzed in this study. Cells were sorted an a FACSAria flow cytometer using standard procedures. The indicated early and late S-phases were isolated for replication timing analysis as described in Methods. Percentages shown are those of total sorted cells collected in each fraction.

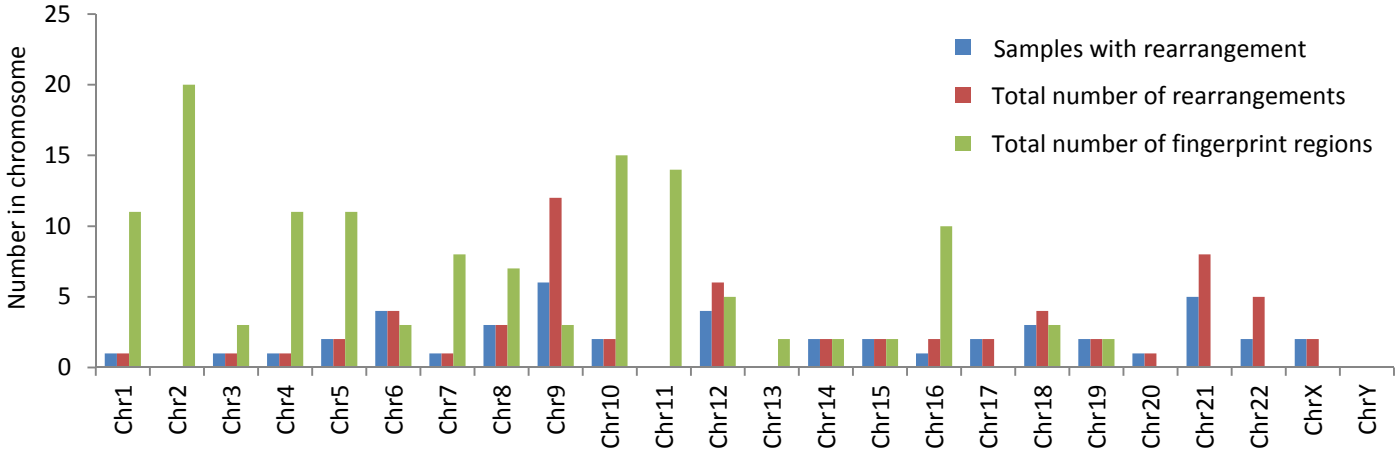

Figure S3. Chromosome distribution of replication fingerprint regions and chromosomal breakpoints. Histogram depicts the number of samples with karyotypic rearrangement, total number of karyotypic rearrangements, and number of fingerprint regions for each chromosome. The number and distribution of all fingerprint regions (leukemia-specific timing changes in Supplementary Table 2) are less clustered into commonly rearranged chromosomes than breakpoints than those in leukemic sample karyotypes.

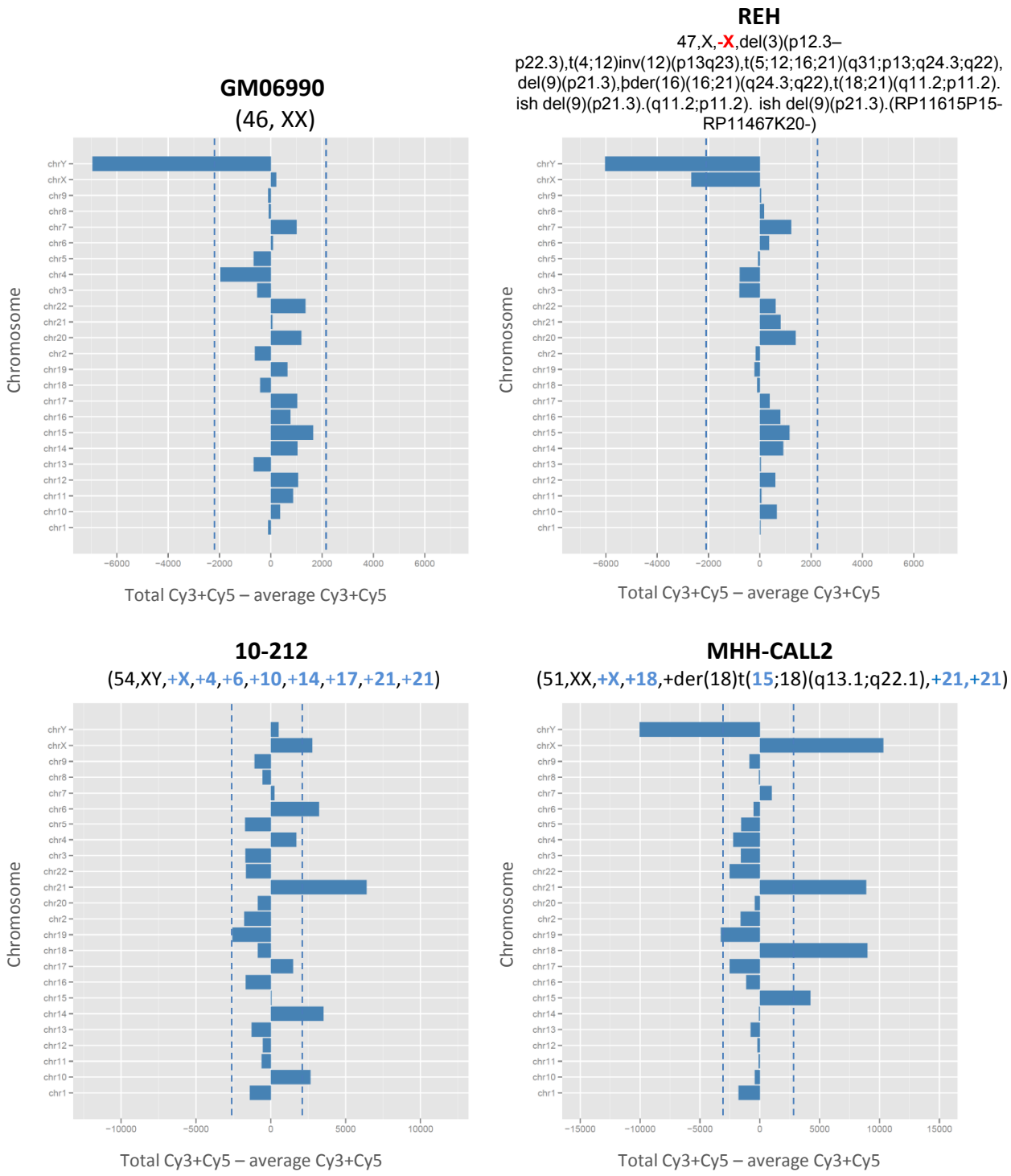

Figure S4. Identification of hyper- or hypodiploid chromosomes by chromosome-wide average Cy3+Cy5 values. Chromosomes with gains or losses in copy number can typically be identified as those with significant deviations from average total intensity on the array, as the number amount of nascent strands hybridized is roughly proportional to the number of copies of each locus. Karyotypic gains and losses of greater than half of a chromosome are highlighted in blue and red, respectively.

Reh karyotype

A

47,X,-X,del(3)(p12.3–p22.3),t(4;12)inv(12)(p13q23),t(5;12;16;21)(q31;p13;q24.3;q22),del(9)(p21.3),þder(16)(16;21)(q24.3;q22),t(18;21)(q11.2;p11.2). ish del(9)(p21.3).(q11.2;p11.2) ish del(9)(p21.3).(RP11615P15-RP11467K20-)

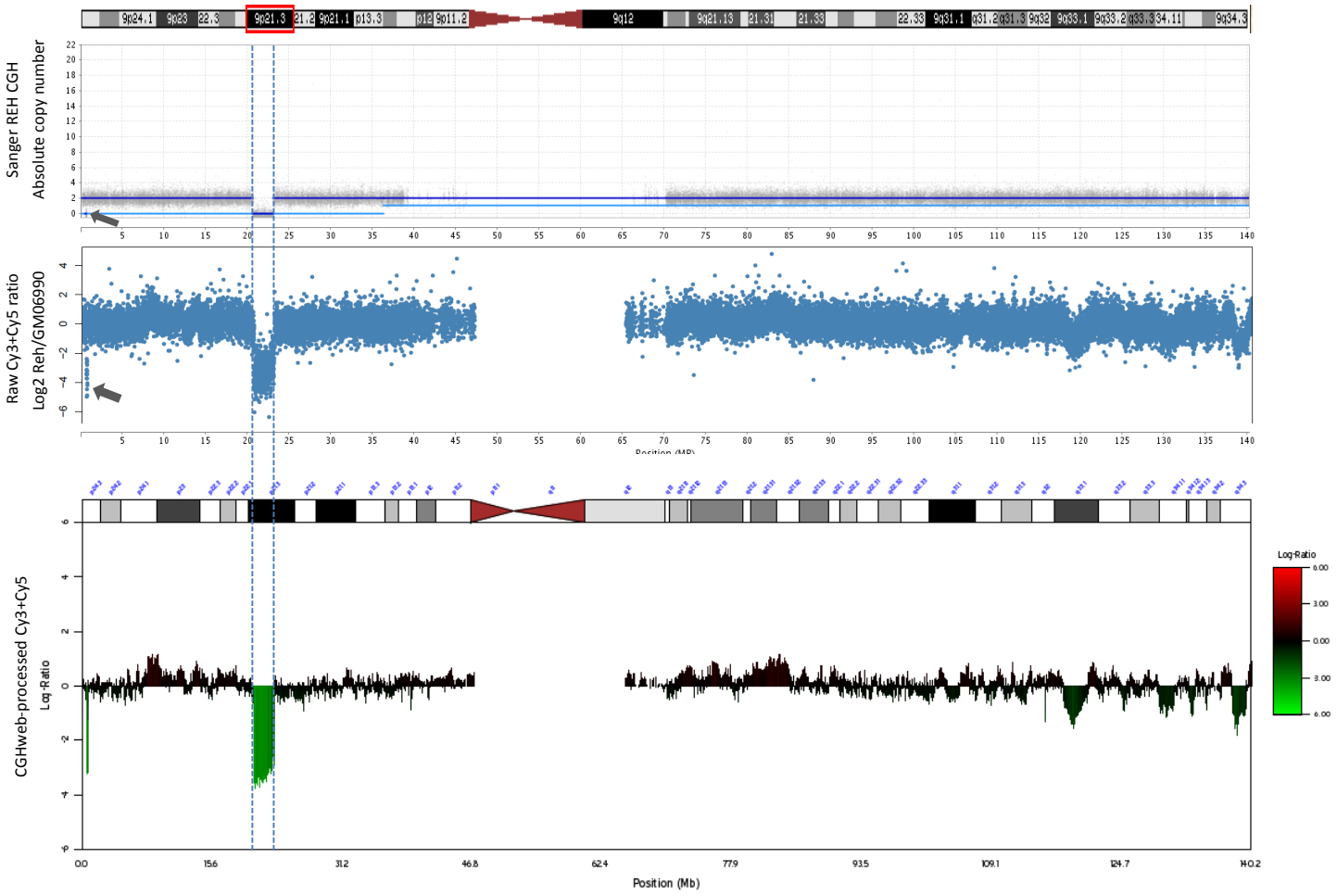

Figure S5. Confirmation of copy number variants in REH using raw replication timing array values. Example of CNVs in chromosome 9 from regions with deletions previously identified from karyotype analysis (del(9)(p21.3); A) and CGH analysis from the Sanger Cancer Genome Project (B). Copy number variations in the leukemic cell line REH are readily detected as abrupt shifts to lower Cy3+Cy5 intensity in REH cells versus a normal karyotype control (C,D). Raw timing array values also detect a small (~20 kb) interstitial deletion in 9p21.3 (arrow in C) in agreement with that detected by CGH (arrow in B).

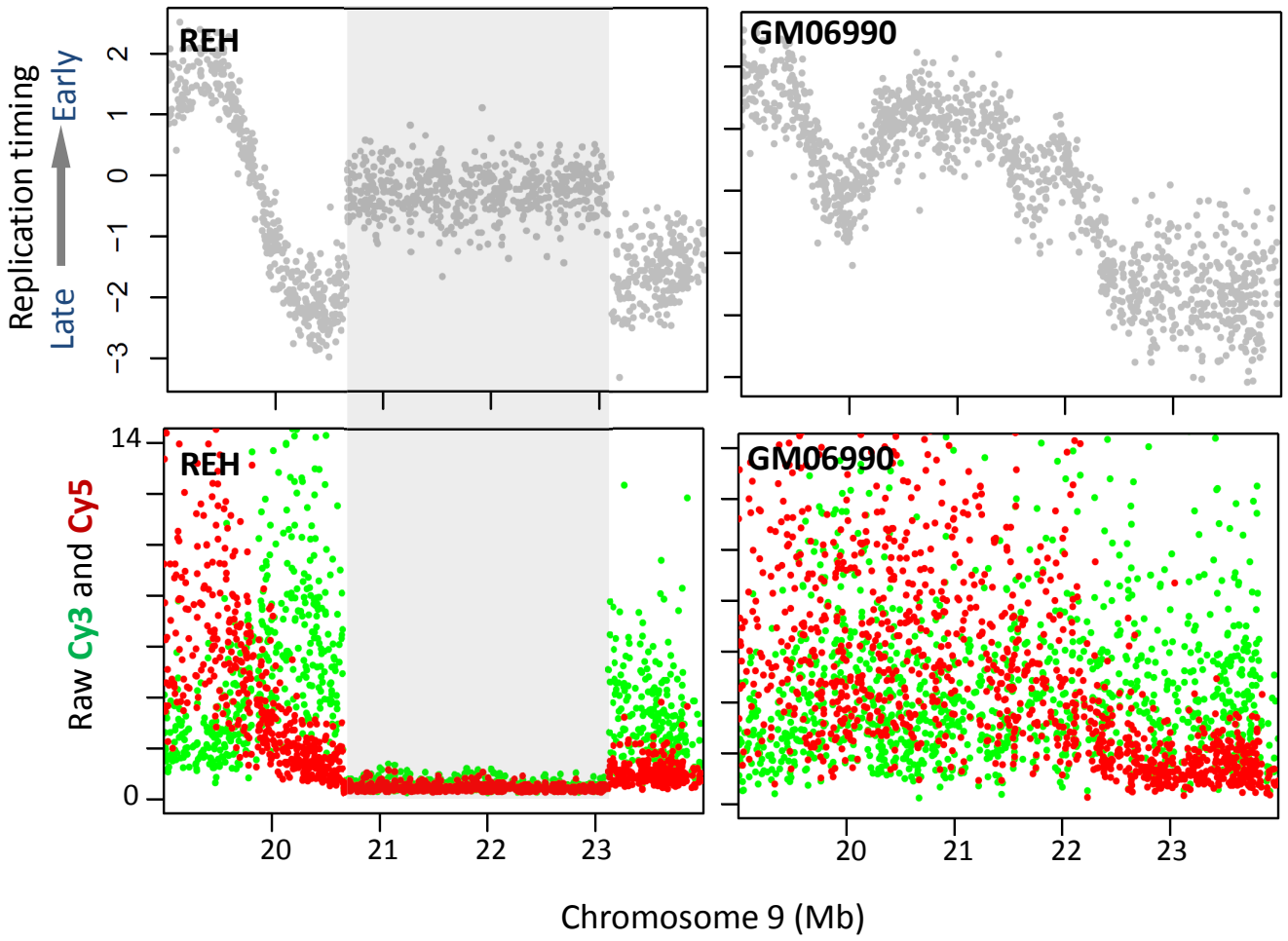

Figure S6. Verification of karyotypically known rearrangements in REH by abrupt changes in replication timing and array intensity. A known deletion in REH on chromosome 9 is readily detected as a shift of replication timing ratios to 0 (indicating no hybridization above background in early or late channels) and as a sharp drop in Cy3 and Cy5 dye intensities to background levels. The corresponding region in B-cell line GM06990 lacks the rearrangement.

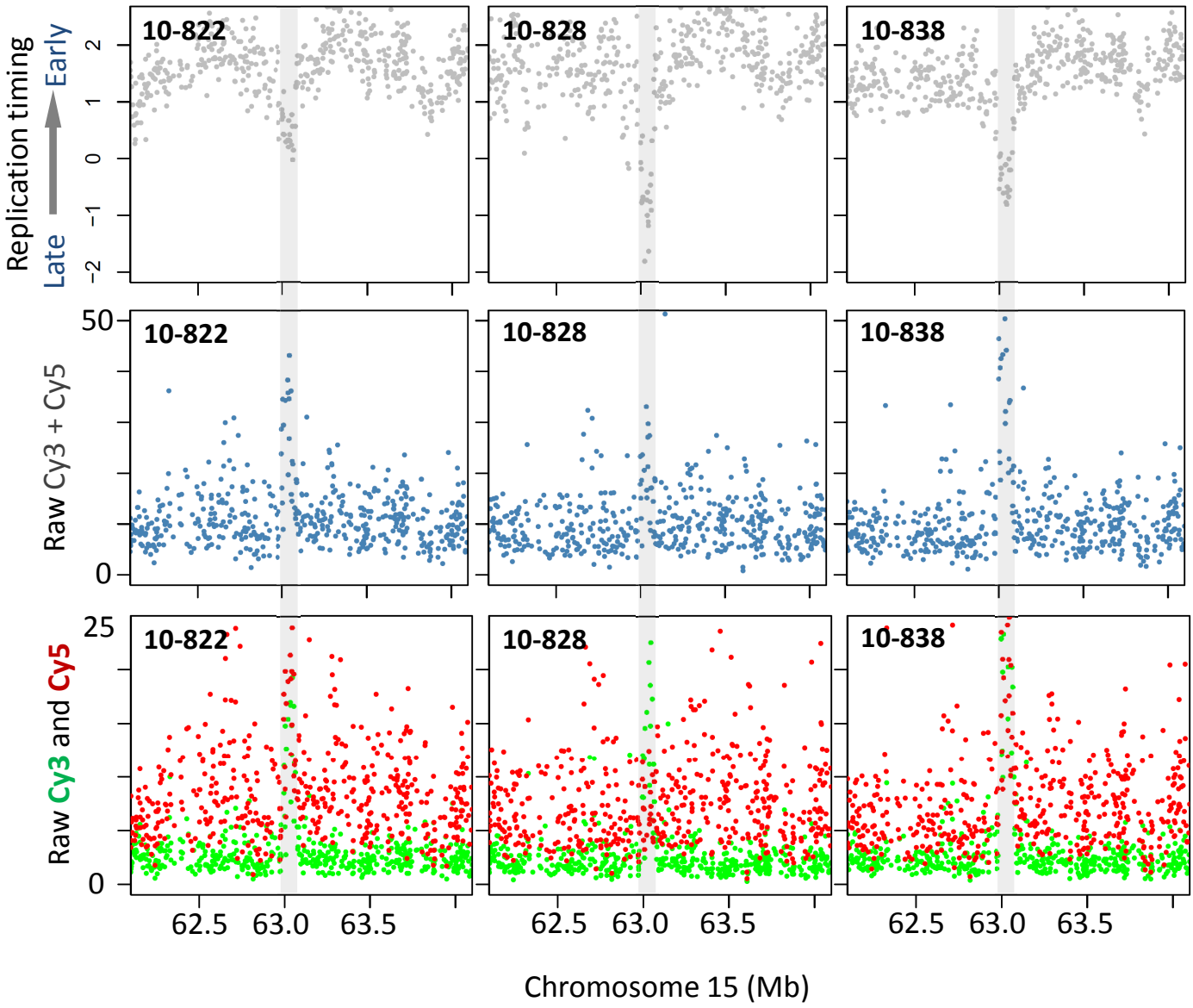

Figure S7. Identification of novel rearrangements in patient samples by abrupt changes in replication timing and array intensity. Novel gains of copy in patient samples 10-822, 10-828, and 10-838 are detected as a sudden increase in the floor of Cy3 and Cy5 raw intensities (bottom profiles, 62 Mb) or by both raw intensities and corresponding abrupt shifts in replication timing (63 Mb).

Detected Not found

A

Patient samples

Lab #	Karyotype	Gains*	Losses*	Translocations	
11-220	55,XX,+X,+4,+6,+10,del(10)(q2?4),+14,+17,+18,+21,+21[18]/46,XX[2]	chr10: 98,000,001-105,700,0001	None	None	
10-820	46,XX,t(9;15)(q13;p1?1.2)[18]/46,XX[2]	None	None	chr9: 70,000,001-70,500,000	chr15: 7,900,001-14,100,0002
11-132	46,XX,t(12;17)(p13;q12)[16]/46,XX[4]	None	None	chr12: 1-14,800,000	chr17: 28,800,001-35,400,000
11-253	45,XY,-20[18]/46,XY[2], including (14q32)/CRLF2 (Yp11.3)			chr14: 88,900,001-106,368,585	chrY: 1,264,869-1,281,529
10-822	45,Y,add(X)(p22.1),add(1)(p3?4),del(6)(q1?1.2q2?2),-9,add(12)(q2?2),t(17;19)(q22;p13.3)[1]/46,XY[24]	chr1: 34,400,001-46,500,0003 chr12: 91,200,001-94,800,0004 chrX: 17,100,001-24,900,0006	chr6: 63,400,001-130,400,0005	chr17: 47,600,001-54,900,000	chr19: 1-6,900,000
10-838	46,XX[26]	None	None	None	
11-221	46,XY,t(11;19)(q23;p13.3)[20]	None	None	chr11: 110,000,001-120,700,000	chr19: 1-6,900,000
10-668	46,XY,del(9)(p13),t(9;22)(q34;q11.2)[12]/46,XY,del(9)(p13),der(9)t(9;22)(q34;q11.2),ider(22)(q10)t(9;22)(q34;q11.2)[4]/46,XY[7]	chr9:129,300,001-140,273,2527	chr9:32,800,001-40,200,0008	chr9: 129,300,001-140,273,252	chr22: 16,300,001-24,300,000
10-828	N/A	N/A	N/A	N/A	N/A
10-356	46,XY,t(6;9)(p23;q34),del(15)(q11.2q15)[2]/45,sl,-Y[cp4]/46,sdl1,+4[5]/47,sdl2,+13[5]/46~47,sdl2,+mar1[cp3]/48,sdl3,+mar1[1][9]		chr15: 18,400,001-42,700,000	chr6:13,500,001-15,500,000	chr9:129,300,001-140,273,252

B

Cell Lines

Name	Karyotype	Gains*	Losses*	Translocations	
REH	47,X,-X,del(3)(p12.3–p22.3),t(4;12)inv(12)(p13q23),t(5;12;16;21)(q31;p13;q24.3;q22),del(9)(p21.3),+der(16)(16;21)(q24.3;q22),t(18;21)(q11.2;p11.2). ish del(9)(p21.3).(q11.2;p11.2). ish del(9)(p21.3).(RP11615P15-RP11467K20-)	chr1: 72 Mb chr9: 37 Mb chr14 108 Mb chr18: 22 Mb chr18: 23 Mb chr21: 35 Mb	chr1: 189 Mb chr2: 90 Mb chr2: 99 Mb chr3: 34-61 Mb chr3: 37 Mb chr3: 115 Mb chr4: 20 Mb chr4: 97 Mb chr5: 110 Mb chr5: 130 Mb chr5: 148 Mb chr5: 180 Mb chr6: 30 Mb chr6: 110 Mb chr7: 40 Mb chr7: 145 Mb chr8: 118 Mb chr9: 1 Mb chr9: 21-23 Mb chr10: 30-36 Mb chr10: 48 Mb chr10: 60 Mb chr12: 10 Mb chr12: 90 Mb chr13: 61-63 Mb chr13: 76-78 Mb chr13: 93 Mb chr14: 22 Mb chr14: 72 Mb chr14: 90-93 Mb chr14: 108 Mb chr16: 84 Mb chr17: 39-41 Mb chr18: 45-46 Mb chr18: 73 Mb chr21: 28-30 Mb chr22: 21-22 Mb	chr4:40,900,001-45,600,000 chr12:1-14,800,000 chr18:17,300,001-23,300,000	chr12:94,800,001-107,500,000 chr21:30,500,001-46,944,323 chr21:6,300,001-10,000,000
RCH-ACV	47,XX,t(1;19)(q23;p13),del(6)(q14.1 q15),+8		chr6:75,900,001-92,100,000	chr1:154,800,001-163,800,000	chr19:1-6,900,000
MHH-CALL2	52,XX,+8,+10,+18,+18,+21,+21 [Alternative: 51,XX,+X,+18,+der(18)t(15;18)(q13.1;q22.1),+21,+21]	chr15: 25,700,001-100,338,915 chr18:1-64,900,000	None	None	None

Figure S8. Detection of known karyotypic rearrangements in leukemic patient samples (A) and cell lines (B). Rearrangements detected as abrupt changes in replication timing ratios or raw array intensities as in Fig. S6 are highlighted in green, while those that did not produce abrupt shifts are highlighted red. A more detailed analysis was possible for cell line REH, for which CGH profiles are available through the Sanger CGP project (http://www.sanger.ac.uk/cgi-bin/genetics/CGP/cghviewer/CghHome.cgi). Whole-chromosome gains and losses were more readily detected by chromosome-wide Cy3+Cy5 (Fig.S4), so only sub-chromosomal rearrangements are examined here.

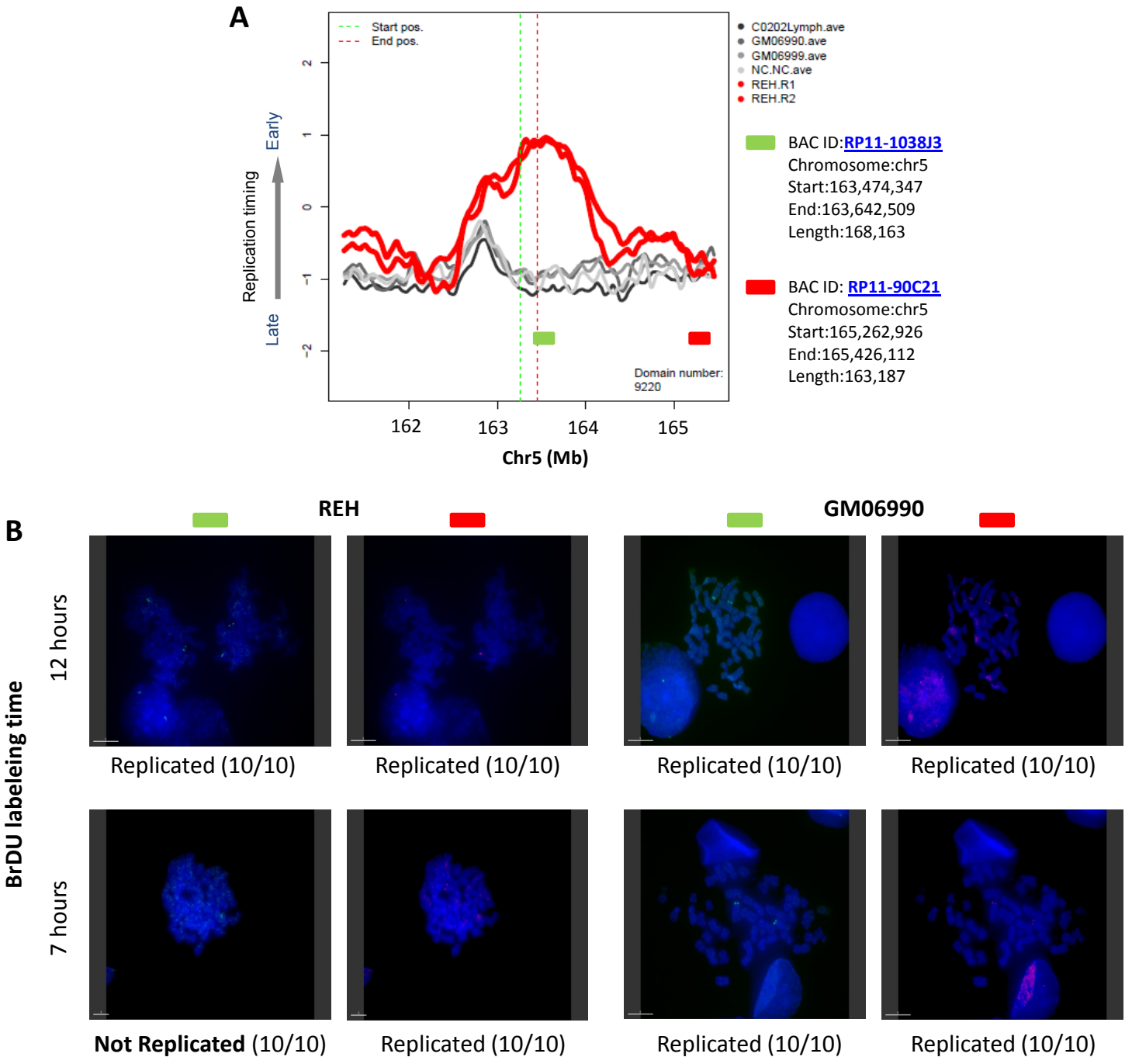

Figure S9. Replication Timing-Specific Hybridization (ReTiSH) to verify an REH-specific replication timing difference. (A) One of the features of the REH-specific replication timing fingerprint is illustrated along with the positions of two FISH probes shown as green and red rectangles. The BAC ID and coordinates of the probes are shown to the right of the graph. (B) ReTiSH relies on the fact that BrdU-substituted DNA is hypersensitive to breakage by UV light. Cells are labeled with BrdU for either 7 or 12 hours and metaphase spreads are prepared. Cells in metaphase will have incorporated BrdU either late (7 hour labeling) or throughout most of S (12 hour labeling). Metaphase spreads are treated with UV light to destroy BrdU-substituted DNA strands and FISH probes are hybridized without denaturation. As expected, both probes hybridize to metaphase spreads of both cell lines with a 12-hour labeling. The non-fingerprint control probe hybridizes to metaphase spreads from both cell lines with a 7-hour label, demonstrating its late replication in all cases. However, the fingerprint probe hybridized to GM06990 but not REH metaphases with a 7 hour labeling, indicating that it is replicated early in S phase. 10 metaphases were counted for each condition and 100% gave the indicated result.

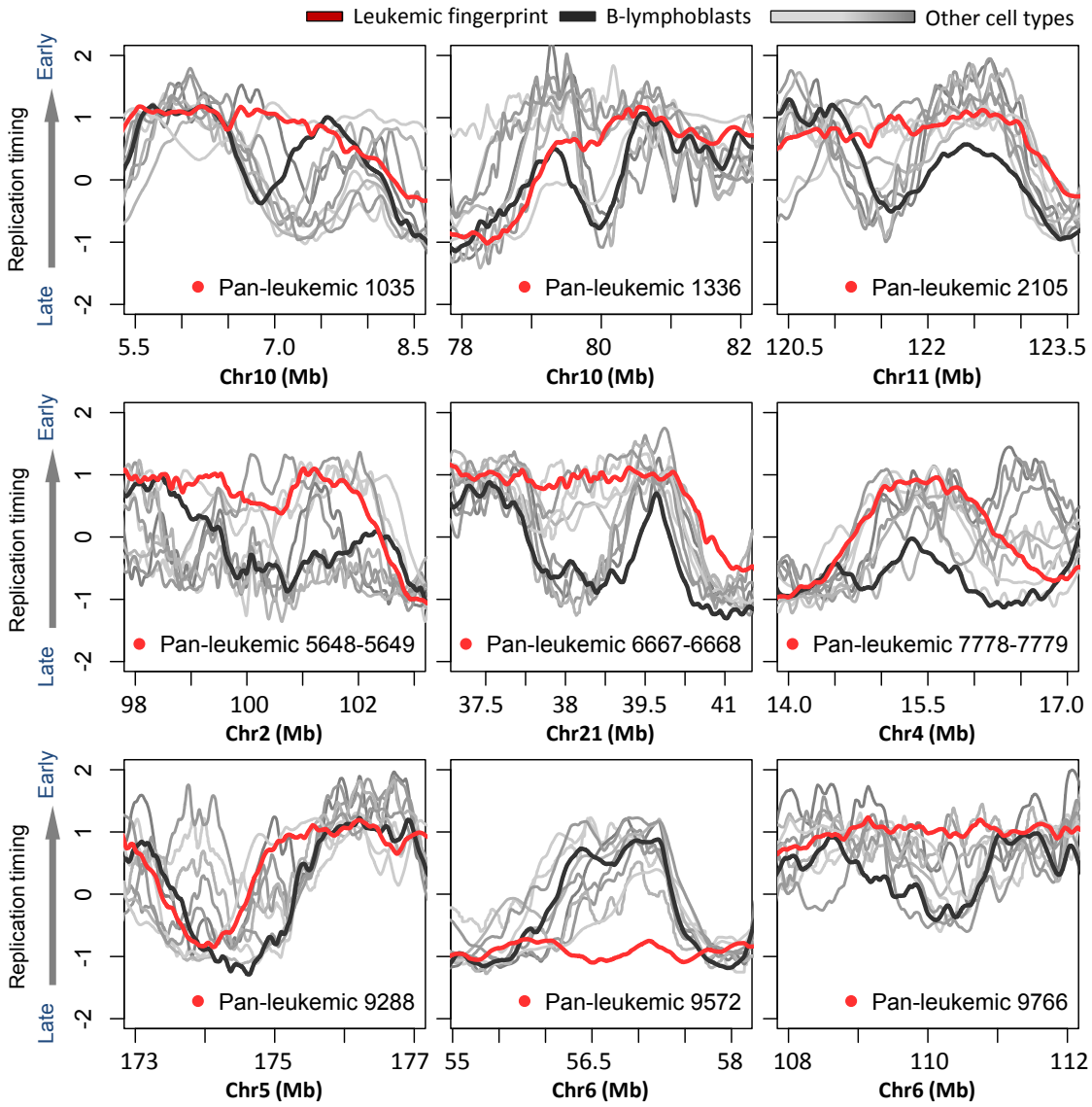

Figure S10. Additional developmental boundary examples. Averages of leukemic profiles (red) are examined in pan-leukemic fingerprint regions. Except for region 9572, which remains late in leukemic profiles, each region aligns to both boundaries and timing established by other cell types in development (grey), but has timing values distinct from the average profile of normal B-cells (black).