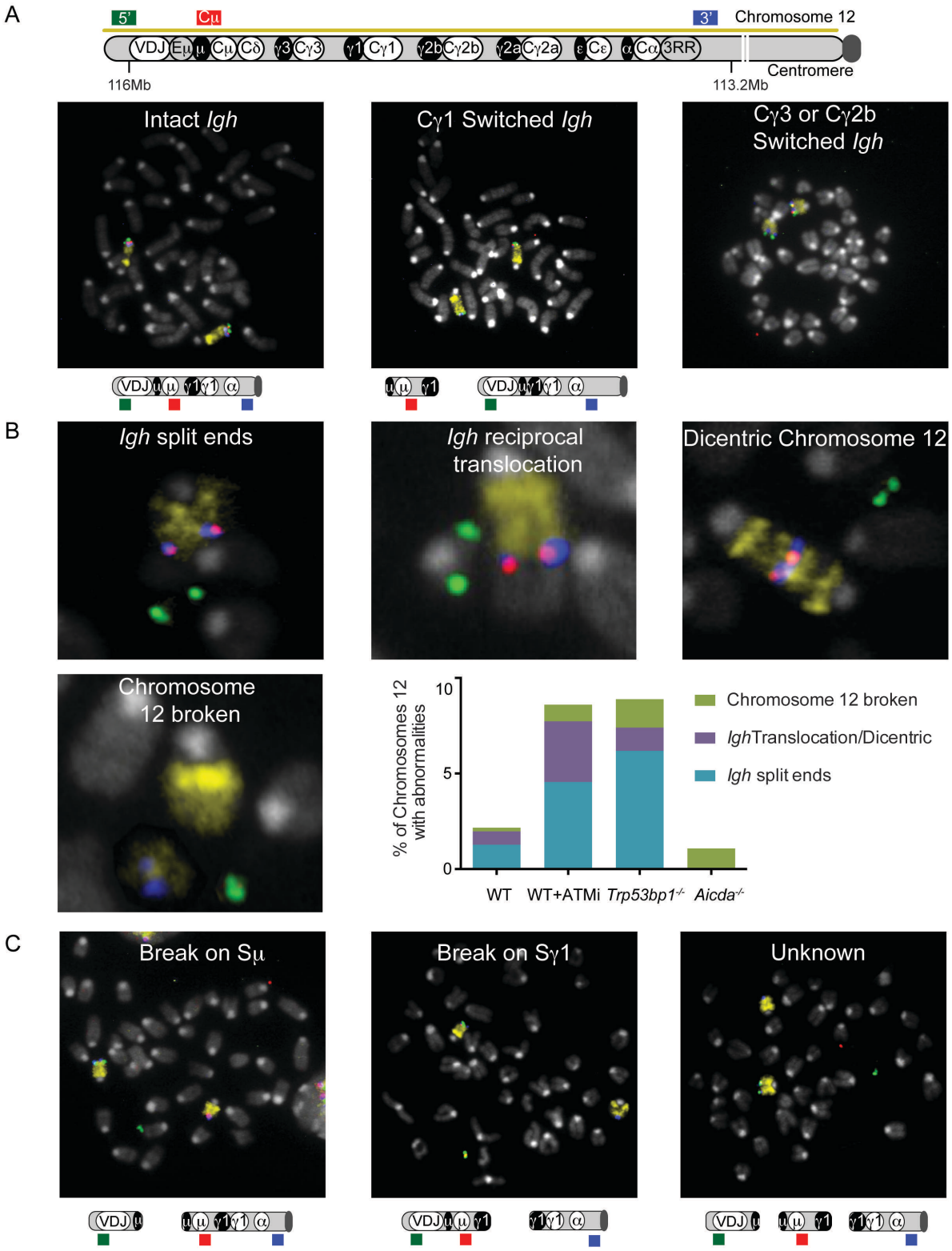

Figure S1. Examples of switching, chromosomal abnormalities and DSB break order.

Related to Figure 1 and 2.

(**A**) Scheme of the *Igh* locus on mouse chromosome 12 showing the location of the differentially labeled probes used in metaphase-FISH analyses (Top panel). Cells used in **Figure 1** and **2** showing examples of intact, Cγ1 as well as Cγ3/Cγ2b switched *Igh* alleles

(bottom panel). (**B**) Examples of chromosomal abnormalities identified in activated B cells. Plot represents the average frequency of each chromosomal defect across replicate experiments. The percentage was calculated as a proportion of the total number of mouse chromosomes 12 analyzed (see **Table S2** for details). (**C**) Cells used in **Figure 1 and 2** showing examples of *Igh* alleles with breaks introduced first in Sμ, Sγ1 or the category classified as 'Unknown'.

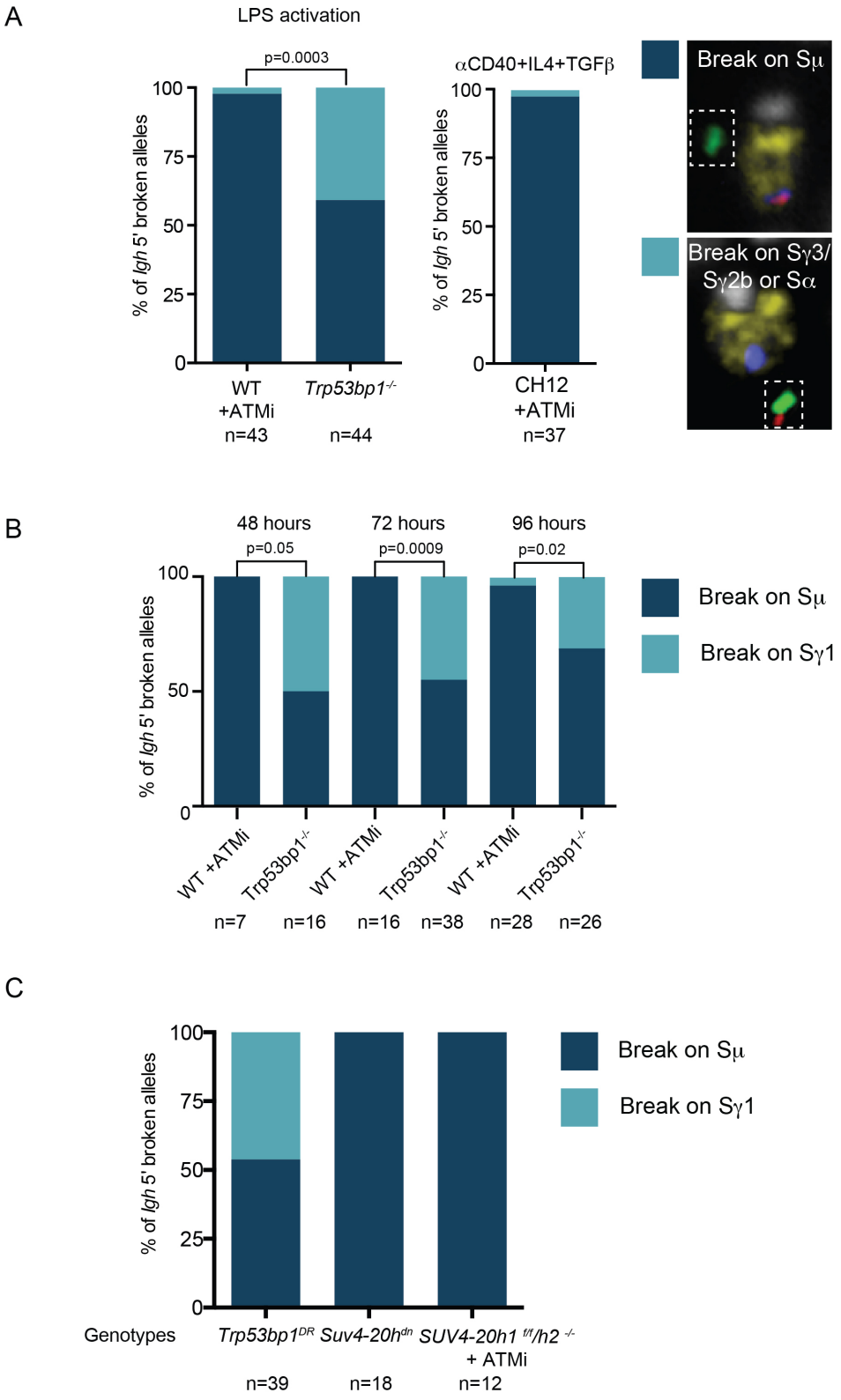

Figure S2. Dynamics of break introduction in different activation conditions and in cells deficient for H4K20me2/m3 or deficient for 53BP1 Tudor domain.

Related to Figure 2.

Igh alleles where location of the first break can be determined were divided into break first on Sμ or Sγ1 (or Sγ2b/Sα) categories for the different mutants or treatments. Significance was

calculated using Fisher's exact test. **Table S3** details the number of replicates and alleles analyzed. Plots represent the average frequency of each category across replicate experiments. Numbers show the amount of *Igh* alleles where break order could be determined. (**A**) Metaphase analysis of WT and 53BP1 deficient cells treated with LPS for 72 hours. CH12 cells were treated for 48 hours with aCD40, IL4 and TGF-β. Broken signals were placed next to chromosome of origin for clarity (**B**) Metaphase analysis of WT cells treated with ATMi and 53BP1 deficient B cells. Cells were culture for either 48, 72 or 96 hours with αCD40 and IL4. (**C**) Metaphase analysis of B cells originating from mice carrying the 53BP1DR mutation and mice deficient for SUV4-20H enzymes. As a control, cells deficient in both SUV4-20H enzymes (*Suv4-20^{dn})* were compared to cells carrying an intact copy of SUV4-20H1 (*Suv4-20h1$^{f/f}$/h2$^{-/-}$*) that were treated with ATMi to increase the frequency of *Igh* chromosomal abnormalities.

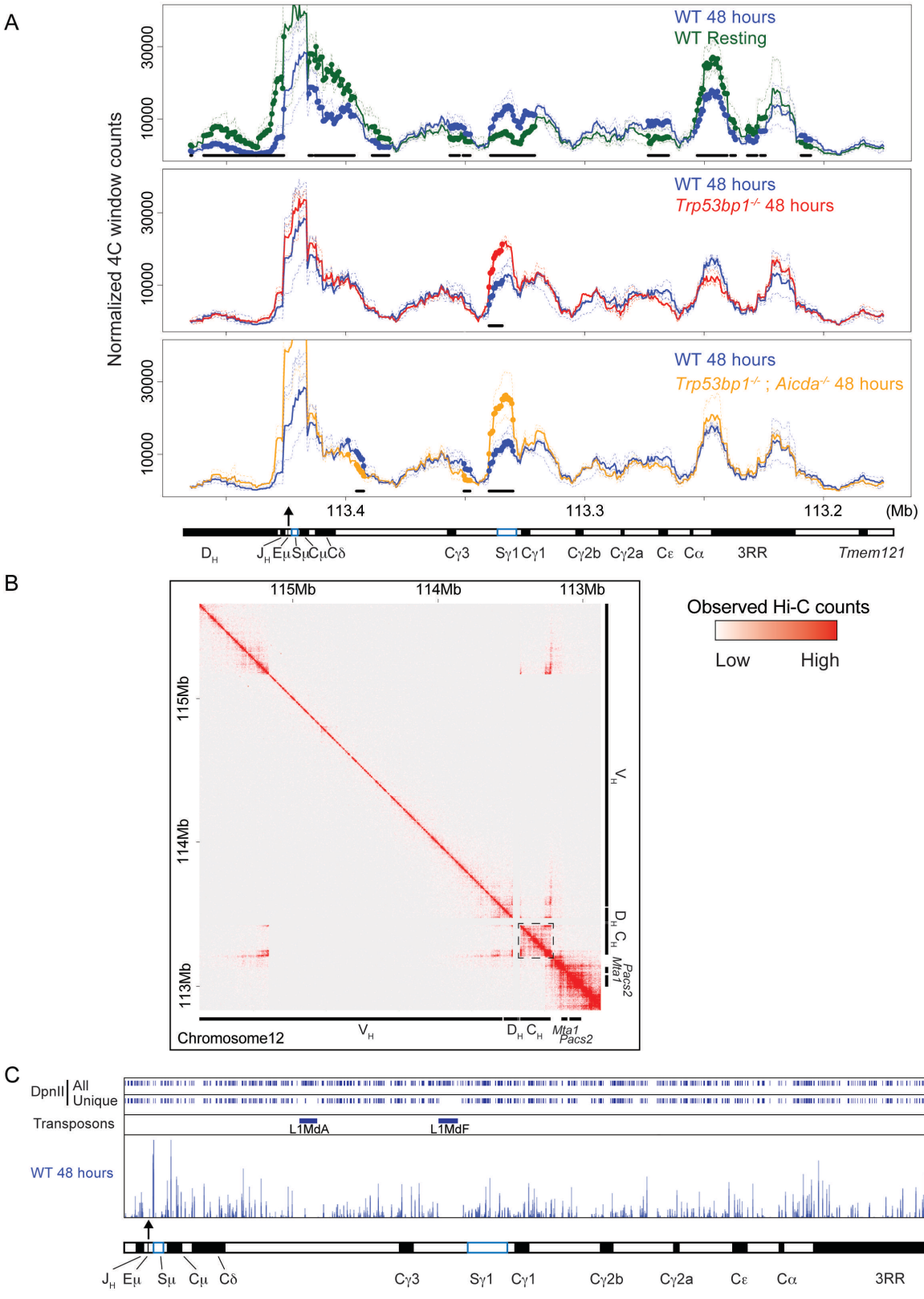

Figure S3 Dynamics of *Igh* chromatin organization during CSR.

Related to Figure 3.

(**A**) 4C-Seq using a bait (black arrow) located on the Eμ enhancer. Lines represent the average 4C-signal across replicates. Dashed lines represent the signal from each replicate.

Colored circles represent windows that are significantly different (adjusted p-value < 0.05) for the two comparisons shown. For easier visualization black bars below the lines represent consecutive windows that are statistically different for the two conditions shown. For simplicity only μ and γ1 switch regions are shown. (**B**) Hi-C data from the CH12 in situ combined track described in (Rao et al., 2014). Dashed square represents the *Igh* constant region (C_H), which is insulated from interactions with genes downstream of *Igh* and with the D_H and V_H regions. (**C**) Mappability of the *Igh* constant region. Top panel shows all 24bp fragments surrounding DpnII sites across the *Igh* constant region in the mm10 genome. The second panel shows the unique fragments flanking DpnII sites on the *Igh* constant region that are used for mapping. Third panel shows the location of transposable elements, which are repetitive regions that cannot be used for 4C-Seq mapping. Bottom panel shows raw 4C-seq signal demonstrating how most of the *Igh* constant region can be mapped using 4C-Seq including the switch regions.

Table S1. Percentage of cells expressing IgG1 as measured by FACS and by metaphase-FISH analysis.

Related to Figure 1.

The increased frequency of switching identified by metaphases is likely related to the fact that some cells identified as switched might have only recombined the unrearranged V(D)J *Igh* allele. Cells with one or two Cμ probes deleted were both classified as switched. Switching was measured after 72 hours of activation.

Genotype (n)	FACS (%)	Metaphases (%)
WT (9)	19.9 ± 3.8	21.5 ± 10.5
Aicda$^{-/-}$ (2)	0%	0%

Table S2. Chromosomal abnormalities upon activation.

Related to Figure 1.

Percentage represents the sum of chromosomal defects observed in each replicate divided by the total number of chromosomes 12 analyzed.

	WT			**WT+ATMi**			***Trp53bp1***$^{-/-}$			***Aicda***$^{-/-}$		
	rep1	rep2	%	rep1	rep2	%	rep1	rep2	%	rep1	rep2	%
Igh split ends	9	13	1.4	42	21	4.6	86	47	6.2	0	0	0
Igh Translocation / Dicentric	5	6	0.7	30	14	3.2	19	7	1.2	0	0	0
Chromosome 12 broken	2	1	0.2	7	5	0.9	13	19	1.5	2	1	1.1
Total Chromosomes 12	756	818		806	578		1194	956		124	154	

Table S3. Analysis of the location of the first break.

Related to Figures 2 and 3.

Percentage of first break in Sμ or Sγ1 is calculated by summing the occurrence for each category across all replicates and dividing by the sum of all alleles across replicates where the location of the first break can be determined (Total DSB origin - this represents n in the figures). Unknown refers to *Igh* alleles where break location cannot be determined and its percentage is calculated in comparison to all *Igh* alleles identified with broken ends (Total Split *Igh*). The Total Split *Igh* category is the result of the sum of Total DSB Origin and Unknown categories. The Total *Igh* alleles category refers to the total number of chromosomes 12 analyzed for each replicate.

Supplemental Experimental Procedures

Mice

All mice used have been previously described. Mice deficient for: AID (Muramatsu et al., 2000), ATM (Barlow et al., 1996), 53BP1 (Ward et al., 2003) and H2AX (Bassing et al., 2003) as well as mice expressing the $53BP1^{DR}$ (Panier and Durocher, 2013) and $NBS1^{hypo}$ (Williams et al., 2002) proteins were derived from heterozygous or homozygous breeding of mice carrying the original mutation. As previously described (Di Virgilio et al., 2013), mice deficient for RIF1 were derived from breedings of mice carrying a floxed *Rif1* allele and mice with the $Cd19^{Cre}$ knock-in mutation. B cells deficient for both SUV420H enzymes were obtained by breeding of a mouse line carrying a *Suv420h2* homozygous deletion and floxed *Suv420h1* loci together with mice expressing the Vav^{Cre} transgene (Schotta et al., 2008). Control animals for this experiment were chosen based on absence of the Vav^{Cre} allele, which leads to expression of only the SUV420H1 enzyme, which is able to compensate for the H2 deficiency (Schotta et al., 2008). We refer to these control cells as $Suv4\text{-}20h1^{f/f}/h2^{-/-}$ and cells carrying the Vav^{Cre} transgene are $Suv4\text{-}20^{dn}$. Animal care was approved by Institutional Animal Care and Use Committee. Protocols number is 150606-01 (NYU School of Medicine). Young mice of less than 12 weeks were used in all experiments.

B cell activation

Splenic B cells were purified as previously described (Rocha et al., 2012). For switching to IgG1, cells were plated at $0.25x10^6$ cells/ml in RPMI 10% FCS and activated with 5ng/ml of IL4 (Sigma) and 1µg/ml of αCD40 (Bioexcell). LPS activation to IgG2b and IgG3 was performed using a concentration of 25ng/ml. CH12 cells were grown on the same media supplemented with NCTC-109 and activation media supplemented with TGF-β (0.1ng/ml) (Life Technologies), 5ng/ml of IL4 (Sigma) and 1µg/ml of αCD40 (Bioexcell). ATM kinase inhibition was performed with the KU-55933 (Fisher) inhibitor at 5mM. This was added 24 and 48 hours post activation. DNA PK inhibitor (NU7441-Tocris) was administrated at 1mM also at 24 and 48 hours post activation. Cells were collected at 60-65 hours after activation for metaphase analyses except for **Figure 3c** where the following time points were used (48, 72, 96). For 4C-Seq assays cells were collected 48 hours after activation to ensure cells were activated but had not yet undergone DNA excision. As a quality control switching efficiency was assessed by FACS for each experiment following 72 hours of activation.

Metaphase FISH Assays

The following primer pairs (in 5' to 3' orientation) were used for amplification of the Cμ probe: GGCTAAGTGAGCCAGATTGTGCTGGG and AGCCCTGTGTCCTCAGCCCC; TGGGGCTGAGGACACAGGGC and GCTCTGCTCTGCAGAAGGGAAAAACC; GCAGCTTCCACTTCAGTCCTAGCCC and ACTTCACCTGCCAGAGAGCCG; ACTTCACGGCTCTCTGGCAGG and TGCTTGTAGGGGGAGGGAGC ; TAGCTGACCAGCTCCCCTGGC and ACAGGGGGCCACTAATCATGCC.