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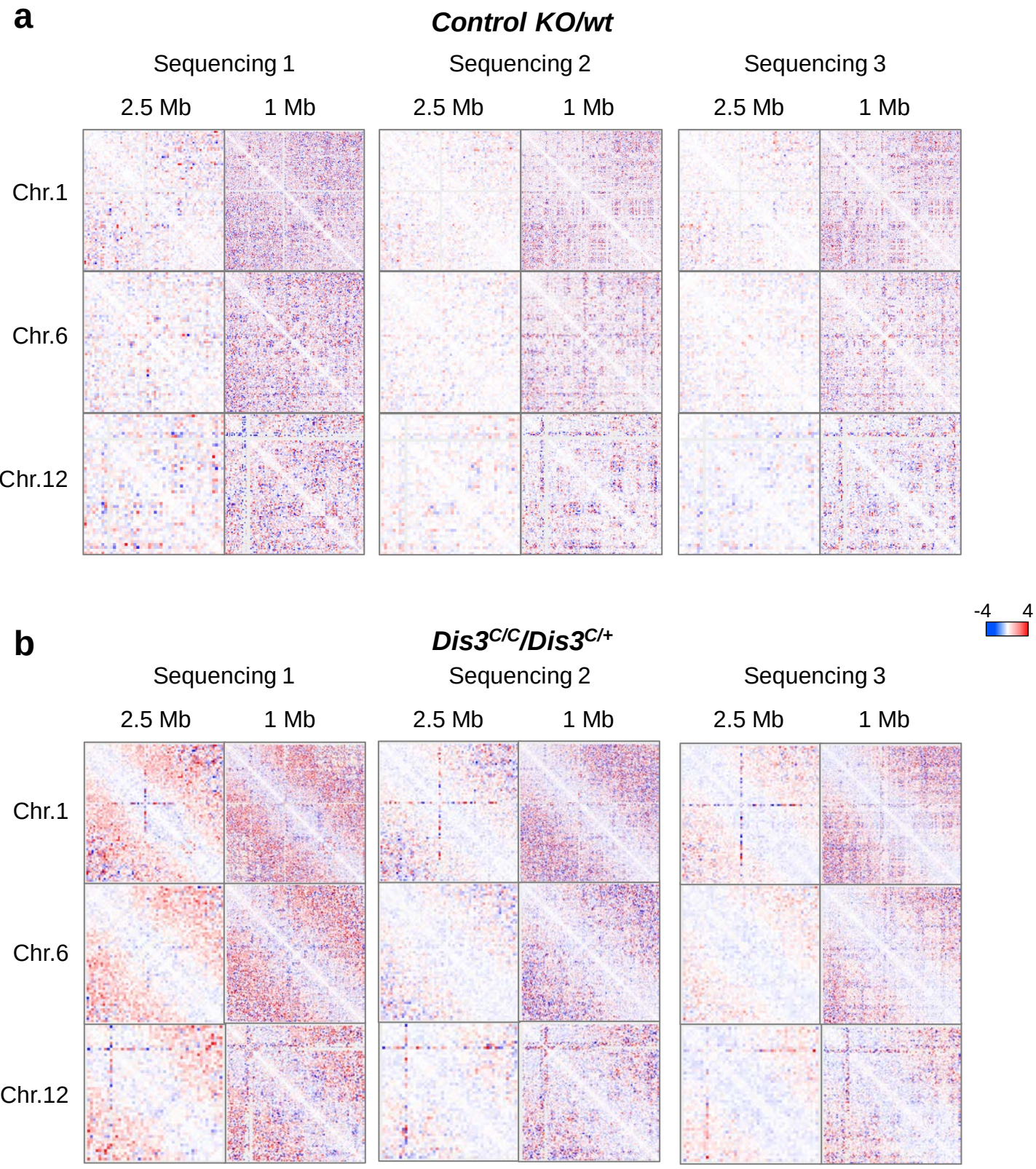
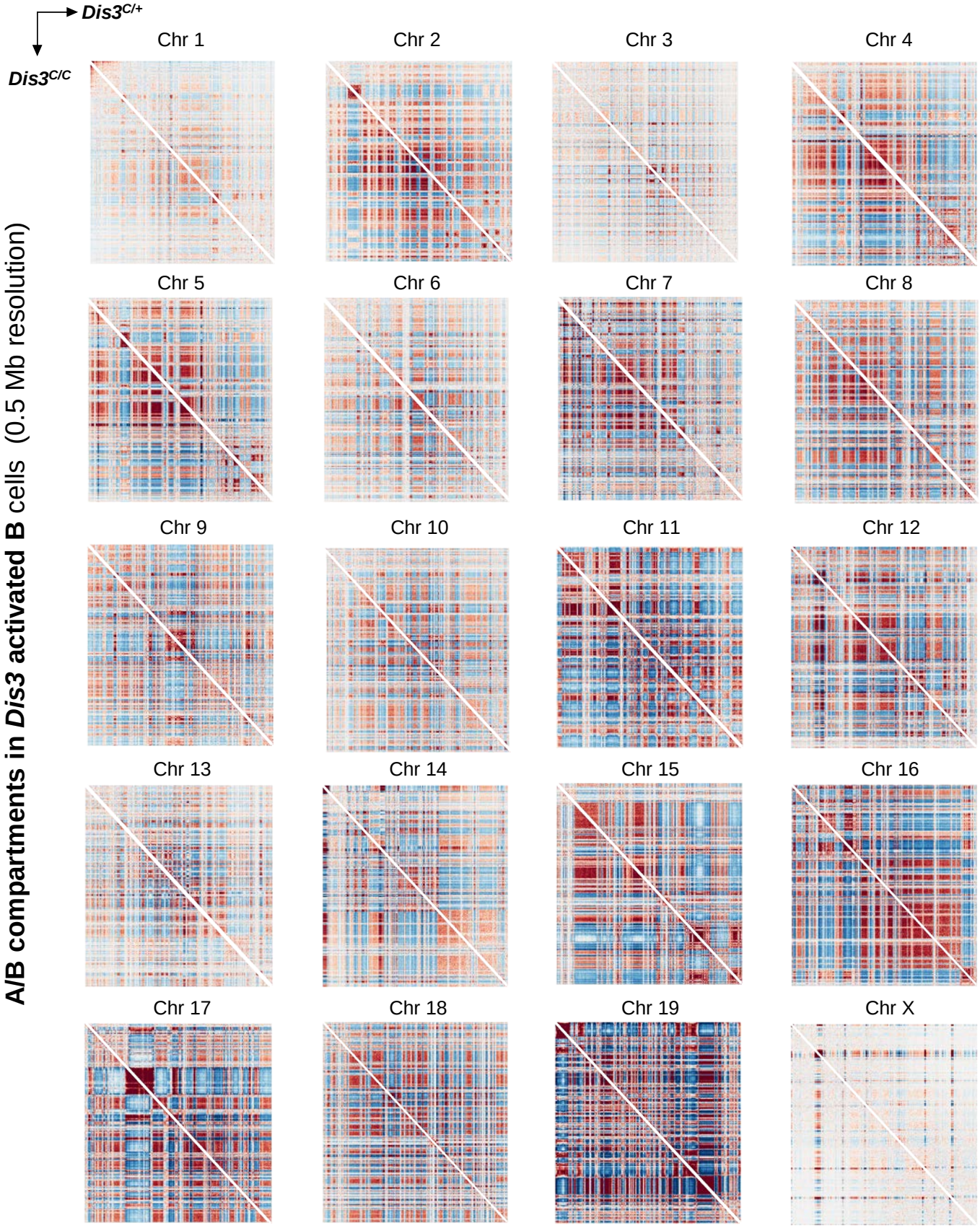
Supplementary information

Noncoding RNA processing by DIS3 regulates chromosomal architecture and somatic hypermutation in B cells

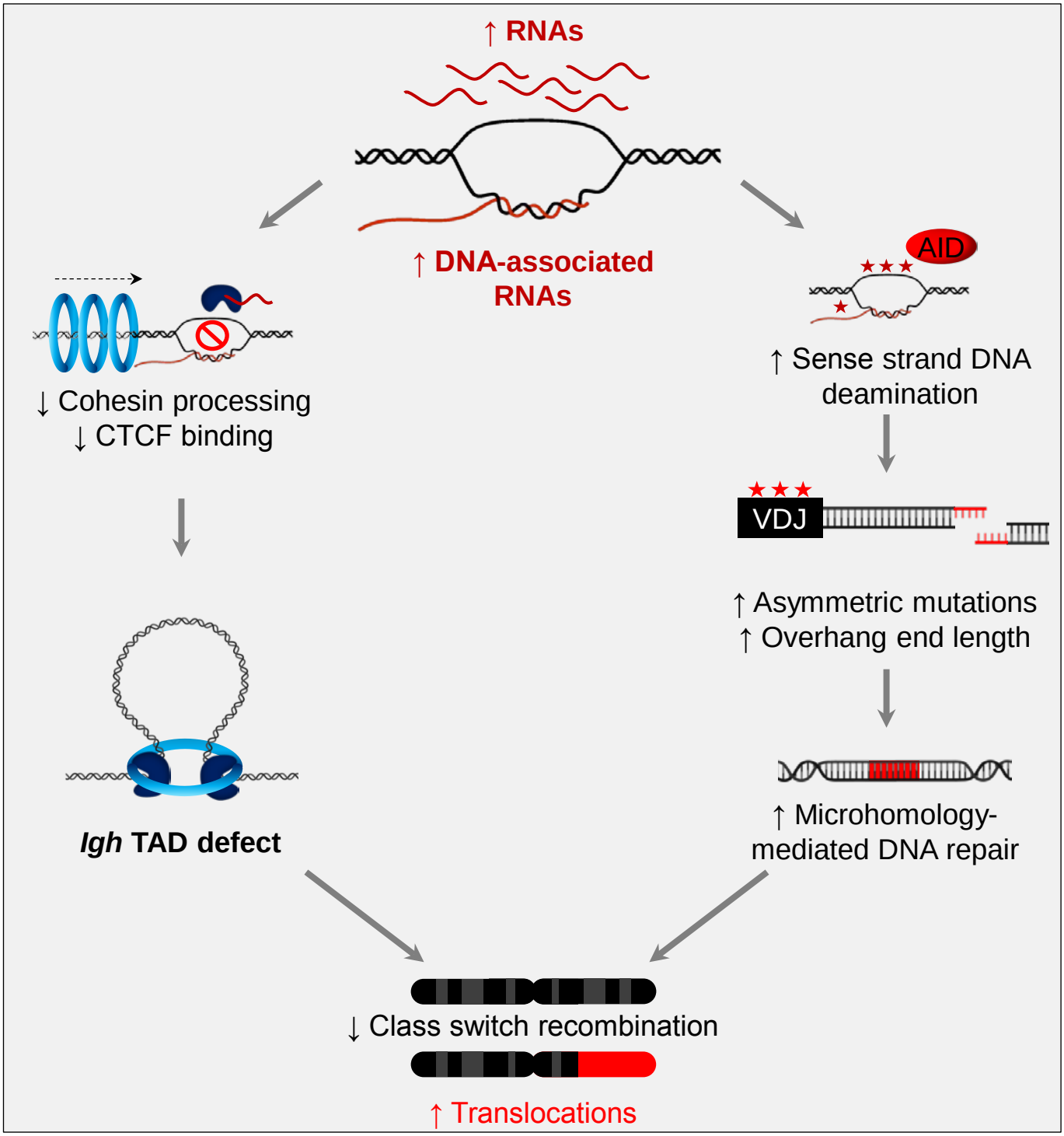
In the format provided by the authors and unedited



Supplemental Figure 1: Reproducible chromosome organization alterations in DIS3-deficient B cells
a. *Control KO/wt* interactions are shown for 3 examples, chromosomes 1, 6, and 12, using different resolution (1 or 2.5 Mb). Random patterns were obtained implying random gain and loss of interaction, equivalent to no change in each sequencing batch from three different biological repeats.
b. Decreased and increased interactions are represented by blue and red colors, respectively. *Dis3*$^{C/C}$/*Dis3*$^{C/+}$ interactions are shown, for 3 examples, chromosomes 1, 6, and 12, using different resolution (1 or 2.5 Mb), with a specific and reproducible pattern of altered genomic interactions in each sequencing batch from three different biological replicates.
Related to **Extended Data Figure 7**.



Supplemental Figure 2: A/B compartmentalization is not affected in DIS3-deficient B cells
A/B compartments in *Rosa*$^{cre/+}$ *Dis3*$^{C/+}$ and *Rosa*$^{cre/+}$ *Dis3*$^{C/C}$ activated B cells at individual chromosomes. Each bin represents a 50 kb bin in the mouse genome (mm9). Compartment A is colored red, and compartment B, blue. Color intensity is scaled by the Pearson's correlation between two regions within the range -0.2 to +0.2. The compartments of *Dis3*$^{C/+}$ and *Dis3*$^{C/C}$ genomes are shown in the upper and lower triangles, respectively.
Note: only one compartment of 4,807 was affected by the absence of DIS3 (0.02%).



Supplemental Figure 3: Proposed model in DIS3-deficient activated B cells
We generated a new *Dis3 COnditinal INversion* allele, allowing the spatio-temporal deletion of DIS3, in activated B cells in this study. DIS3-deficient B cells accumulate RNAs and DNA-associated RNAs which strongly affect two critical aspects of B cell activation. RNAs and DNA:RNA hybrids accumulation decrease the binding of CTCF to its cognate CBEs, and impedes optimal cohesin-mediated loop extrusion and cohesin localization. The cumulative loss of CTCF/cohesin binding and cohesin positioning alter the genome architecture, which strongly affects the *Igh* TAD. This weakness at the *Igh* TAD exposes DIS3-deficient B cells to DNA translocations outside of the *Igh* TAD. DNA-associated RNAs also over-expose sense strand DNA to AID mutagenic activity, starting with deamination of cytosine. This phenomenon leads to asymmetric somatic hypermutation at VDJ gene and increases DNA double strand breaks over-hang length at Sμ and acceptor regions. A bias in microhomology-mediated DNA repair is observed between Sμ and S acceptor regions, or between Sμ and translocation partners. The increased frequency of long DNA over-hang could participate to increase DNA translocations. Note: in human multiple myeloma *DIS3* mutations are associated with translocations implicating the *IGH* locus, as we observed in our model. We propose that the cumulative effect of suboptimal *Igh* TAD formation and decreased DSBs in the absence of DIS3 activity participate to the diminution of CSR events while exposing DSBs to translocate outside of their own TAD.

Supplemental Discussion

DIS3 model system for identifying ncRNA transcription *in vivo*: As *DIS3* is often mutated in GC-derived multiple myeloma plasma cells, we generated appropriated models to study *Dis3* inactivation in activated B cells, prior to their terminal differentiation into plasma cells. Deep RNA sequencing of activated B cells reveals DIS3-substrates, mostly aTSS-RNA, antisense RNAs, eRNAs and lncRNAs, as described previously[4–7], while our analyses unveil a new class of CBE-overlapping RNAs. Importantly, identification of DIS3-sensitive ncRNAs was effective from GC-derived primary cells, demonstrating the robustness of the *Dis3^{COIN}* model to discover ncRNAs *in vivo*. DRIP-sequencing also revealed an unexpectedly massive accumulation of chromatin-associated RNAs in DIS3-deficient activated B cells.

***Igh* somatic mutations and CSR**: With respect to antibody gene diversification, we note that in DIS3-deficient B cells, two separate events occur (a) alteration of *Igh* architecture and (b) changes in mutation distribution at V genes and switch sequences. Deletion of the *Igh* TAD super-anchor leads to a relatively weak decrease in CSR[14,43], while DIS3-deficient B cells have a stronger defect in CSR (~3-fold), suggesting that DIS3's role in controlling AID activity for template strand deamination plays a major and cumulative role with the stability of *Igh* architecture to drive CSR. The question arises about the function of the 3' super-anchor and *Igh* loop extrusion/architecture during CSR. As postulated earlier[13] and illustrated in our model, we speculate that in the conditions of ncRNA and DNA:RNA hybrid accumulation following DIS3 depletion the *Igh* loop extrusion may be perturbed sufficiently to reduce alignment of donor and acceptor switch sequences prior AID-mediated DNA breaks and repair, but may not mimic complete loss of 3'RR function. Molecular dissection of the two functions of DIS3 in controlling *Igh* architecture and AID-mediated deamination provides a unique opportunity to characterize how failure to regulate chromosomal architecture at sites where DNA mutations/breaks are occurring could cause genomic instability.