

Cohesin-dependent chromatin structure at high resolution

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Abstract:

Genome-wide studies to address the three-dimensional structure of different genomes identified a general and conserved organization in topological domains [1]. Recently, we demonstrated that the cohesin complex has an important role in the organization of the topological domains. We showed by 3C-sequencing (3C-seq) and Hi-C that the depletion of cohesin by the proteolytic cleavage of its RAD21 subunit leads to reduction of chromatin interactions, predominantly within topological domains [2].

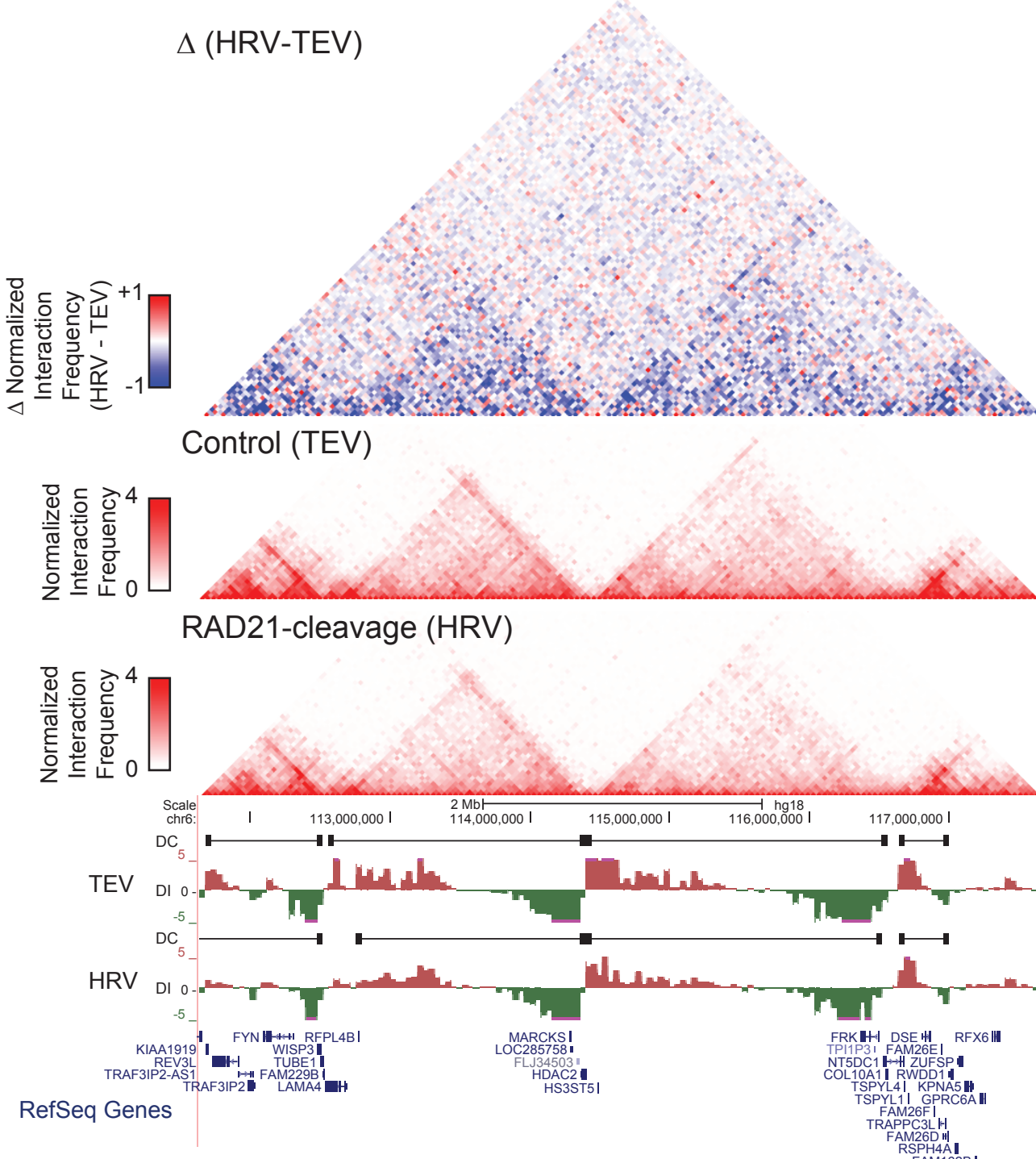
However, the limitation in using a restricted number of DNA fragments as viewpoint for the 3C-seq and the resolution of Hi-C (40kb) are not sufficient to yield information about how the fiber is folded within these domains and which are the detailed effects of the cohesin depletion on the interacting areas.

To address this question we used a high resolution approach called "Targeted Chromatin Capture (T2C)" [3]. T2C provides information of the spatial organization of selected loci at single restriction fragment resolution (2 to 6 kbp). We studied a genomic region of 2.1 Mb on the human chromosome 11 comprising the imprinting loci IGF2/H19 and KCNQ1 to unravel the details.

- Dixon JR. et al. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 485: 376-380.
- Zuin J, Dixon JR. et al. (2014) Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc Natl Acad Sci U S A 111: 996-1001.
- Kolovos P. et al. (2014) Targeted Chromatin Capture (T2C): a novel high resolution high throughput method to detect genomic interactions and regulatory elements. Epigenetics&Chromatin 7: 10.

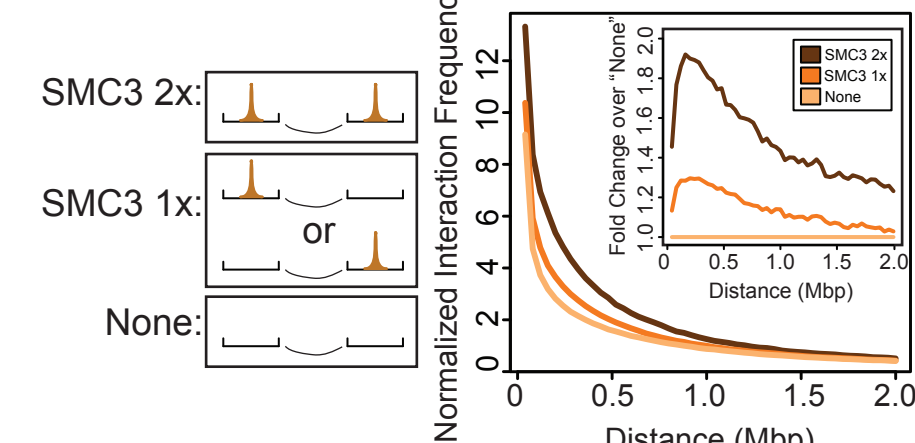
II. Cohesin cleavage leads to global loss of structure

Analysis of changing in chromatin structure after cohesin cleavage by Hi-C



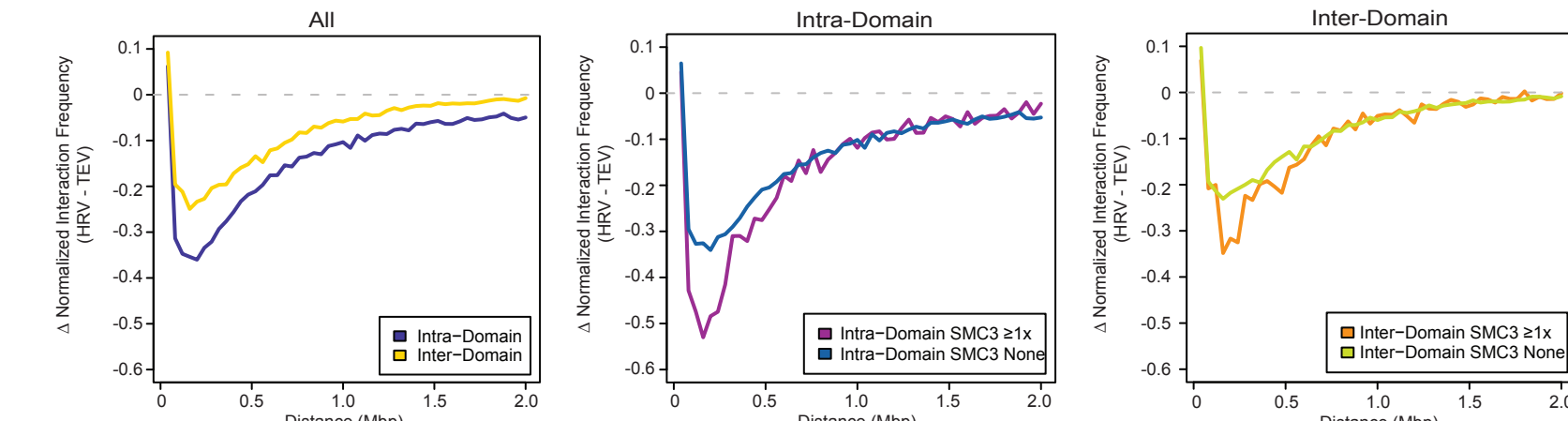
Correlation between interaction frequency and cohesin (SMC3) binding sites

Interactions are more likely present between regions that have at least one SMC3 binding site.



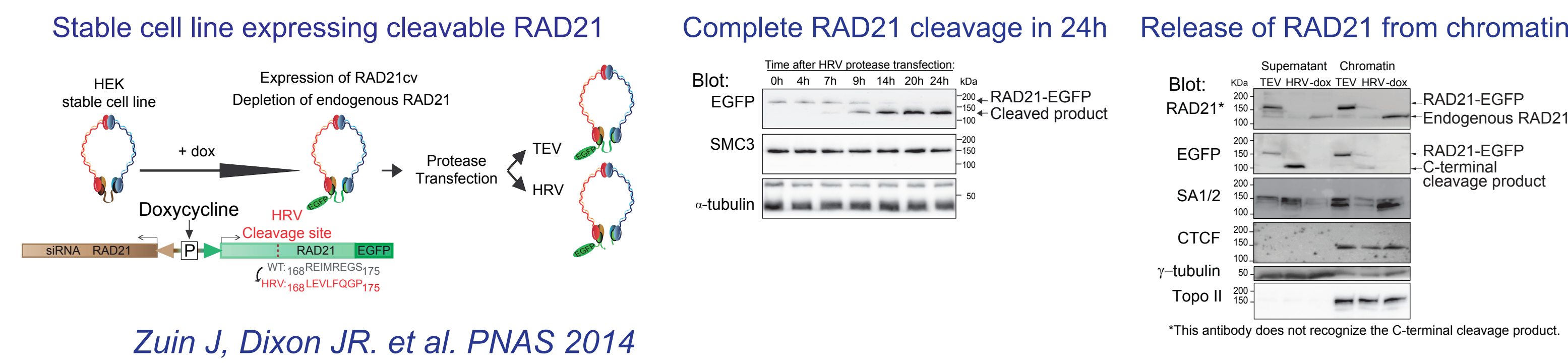
Effect of cohesin cleavage on the topological domains

Cohesin cleavage leads to a general loss of interaction mainly occurring between interacting regions less than 2Mb apart with a maximum in the range between 100-200Kb. The loss of intra- and inter-domain interactions correlates with SMC3 binding.



Zuin J, Dixon JR. et al. PNAS 2014

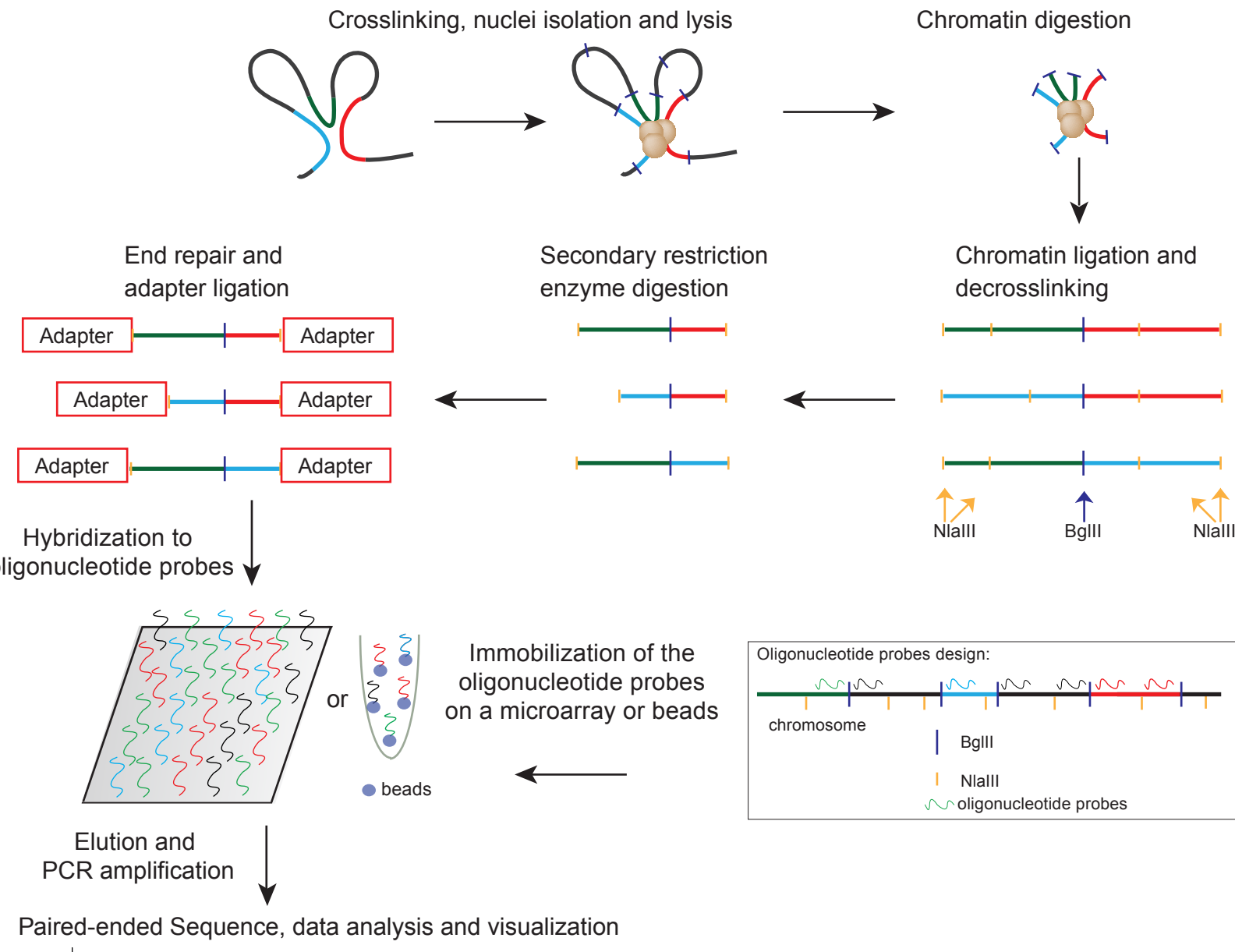
I. Depletion of cohesin by proteolytic cleavage



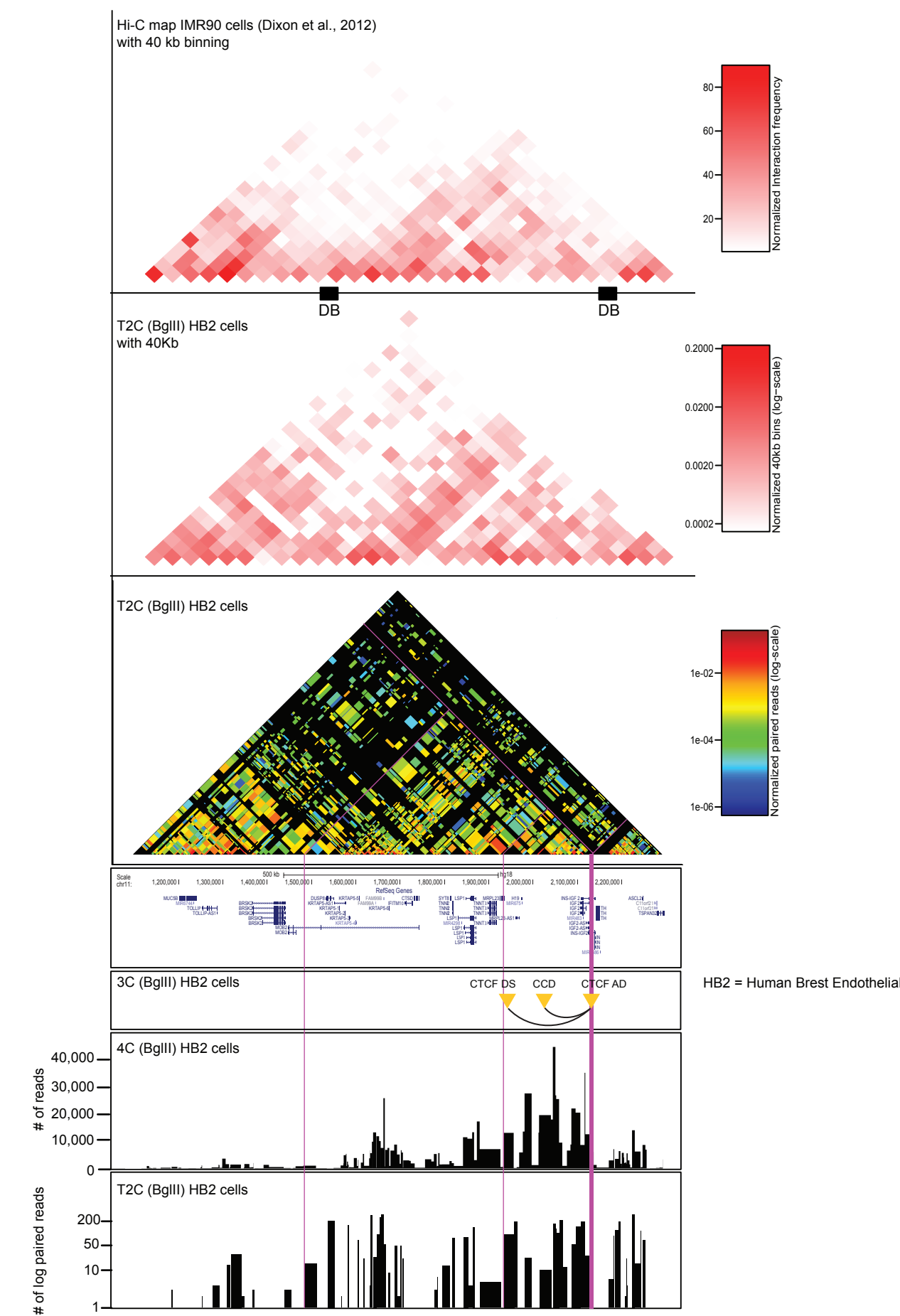
Zuin J, Dixon JR. et al. PNAS 2014

III. Targeted Chromatin Capture (T2C)

Method



Comparison of T2C with other "C" methods



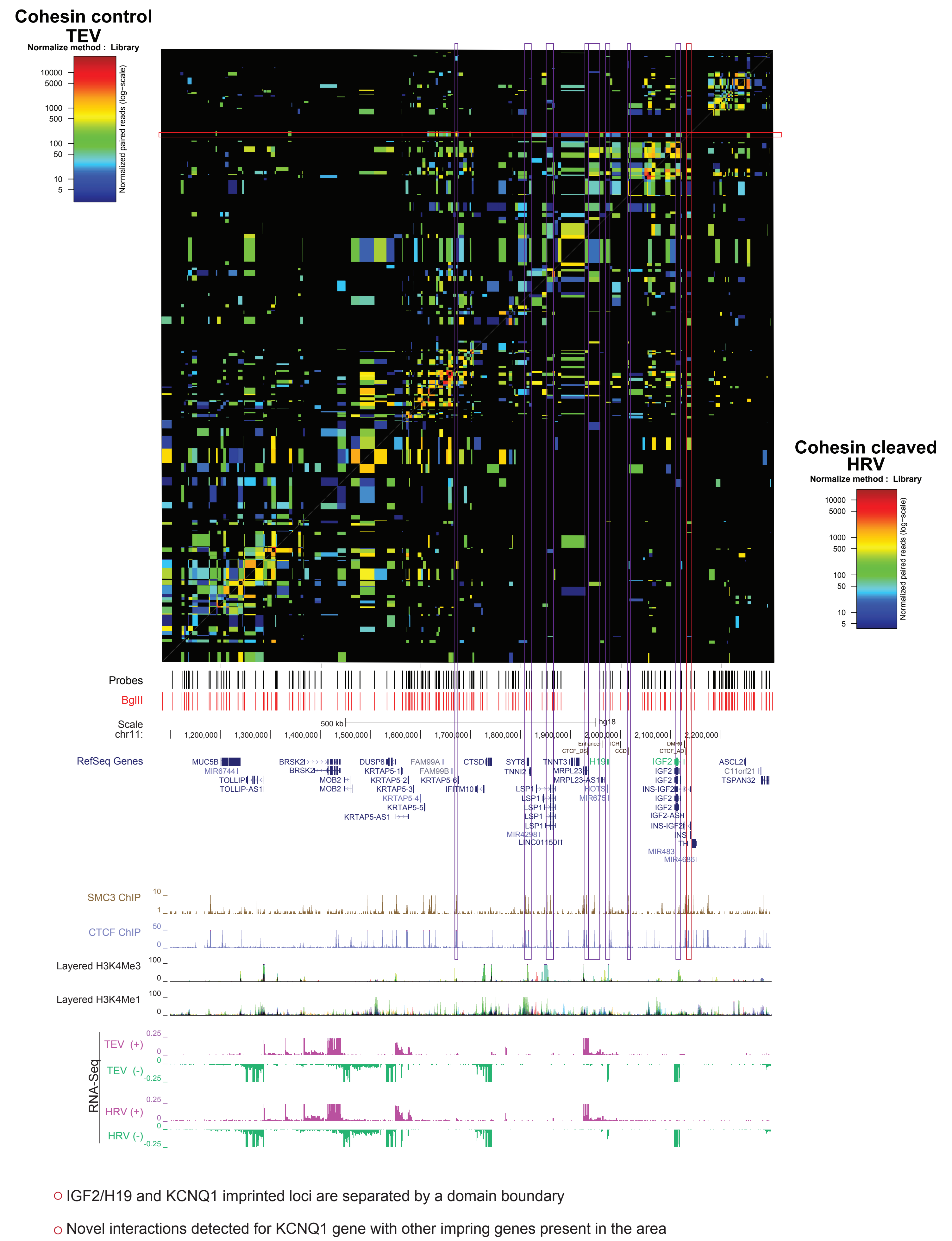
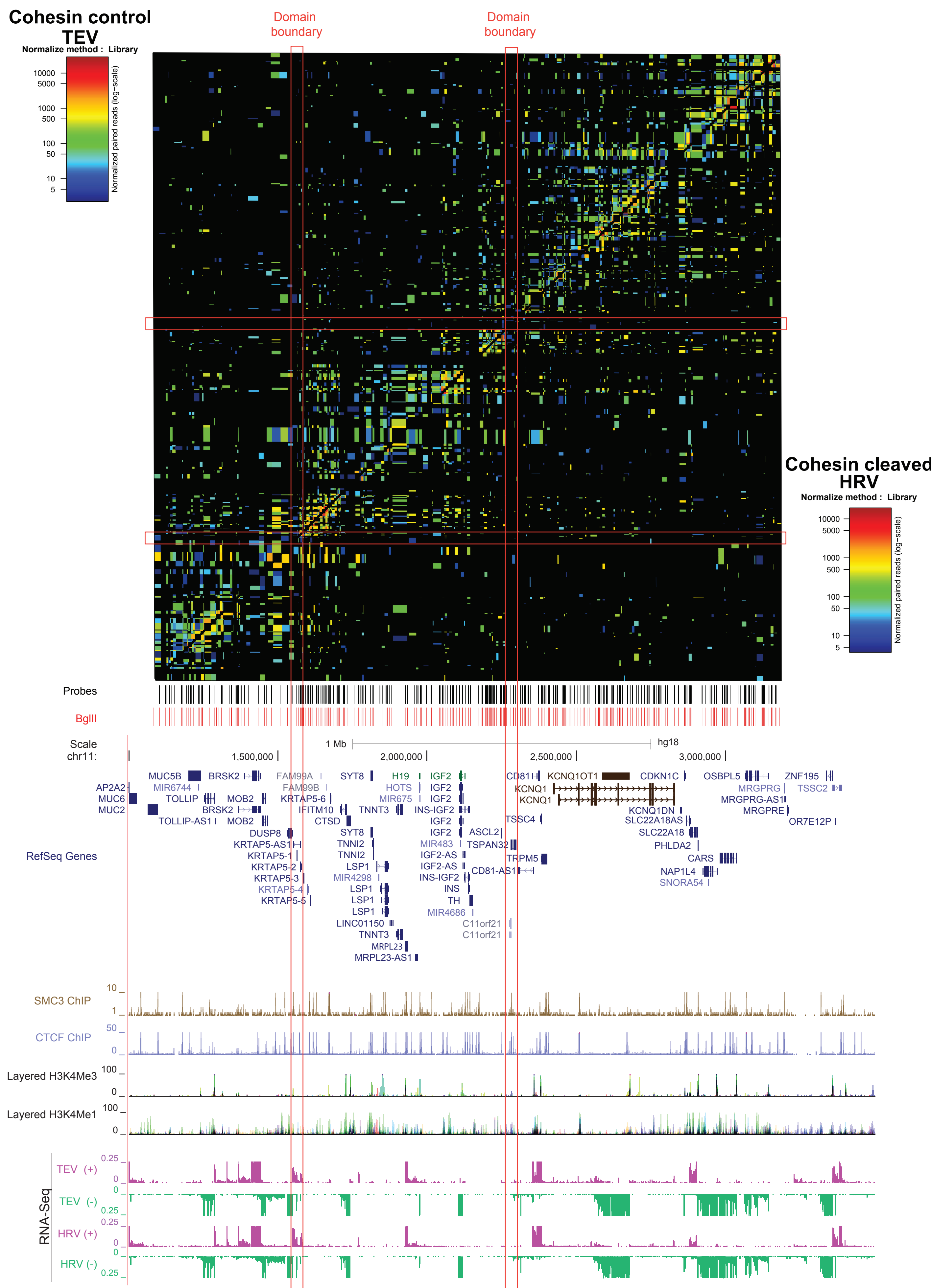
Kolovos P. et al. Epigenetics&Chromatin 2014

- Restriction fragment resolution (~2-6 kb)
- Selected continuous genomic regions
- Selected separated genomic regions (e.g. specific loci)

IV. T2C interacting matrix from cohesin control (TEV) and cohesin cleaved (HRV) cells

Selected genomic region of 2.1Mb comprising IGF2/H19 and KCNQ1 imprinted loci

Zoom-in on the genomic region comprising IGF2/H19 locus



- IGF2/H19 and KCNQ1 imprinted loci are separated by a domain boundary
- Novel interactions detected for KCNQ1 gene with other imprinting genes present in the area
- Cohesin sites often interact with each other

V. Future perspectives

Correlation of interaction frequency and cohesin at binding site level

Analysis of individual binding sites that might be important in forming interactions

Analysis of changing in chromatin structure after cohesin cleavage at restriction fragment level

