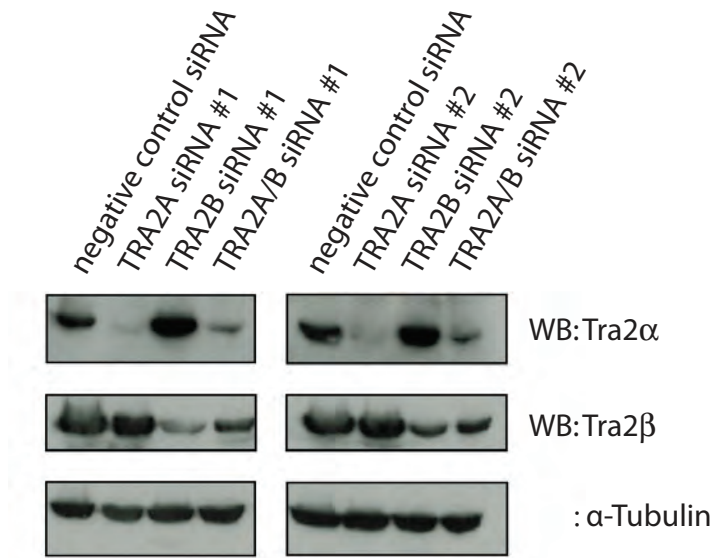
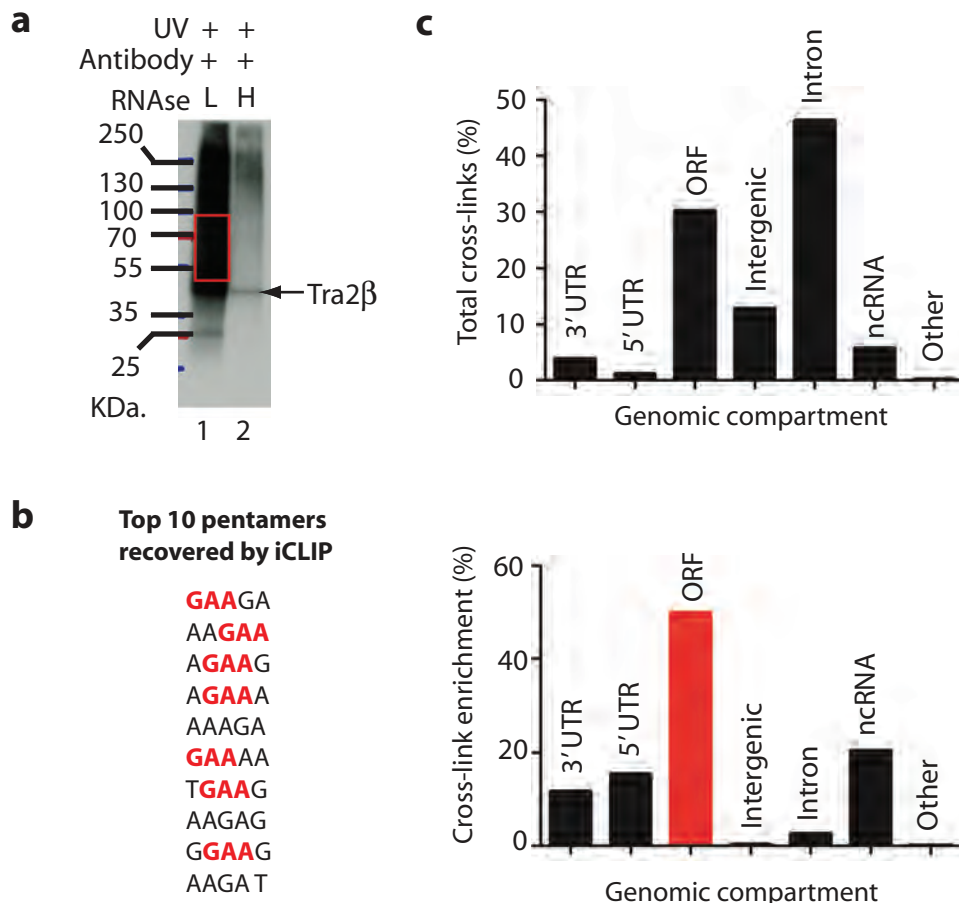


Supplementary Figure 1.



Supplementary Figure 1. Depletion of endogenous Tra2 β results in strong up-regulation of endogenous Tra2 α protein. Efficient protein knockdown was confirmed by Western blot, using 2 siRNA sequences targeting each of the TRA2A and TRA2B mRNAs. Strong up-regulation of Tra2 α was confirmed after independent transfection of MDA-MB-231 cells with two independent siRNAs specific to TRA2B.

Supplementary Figure 2.



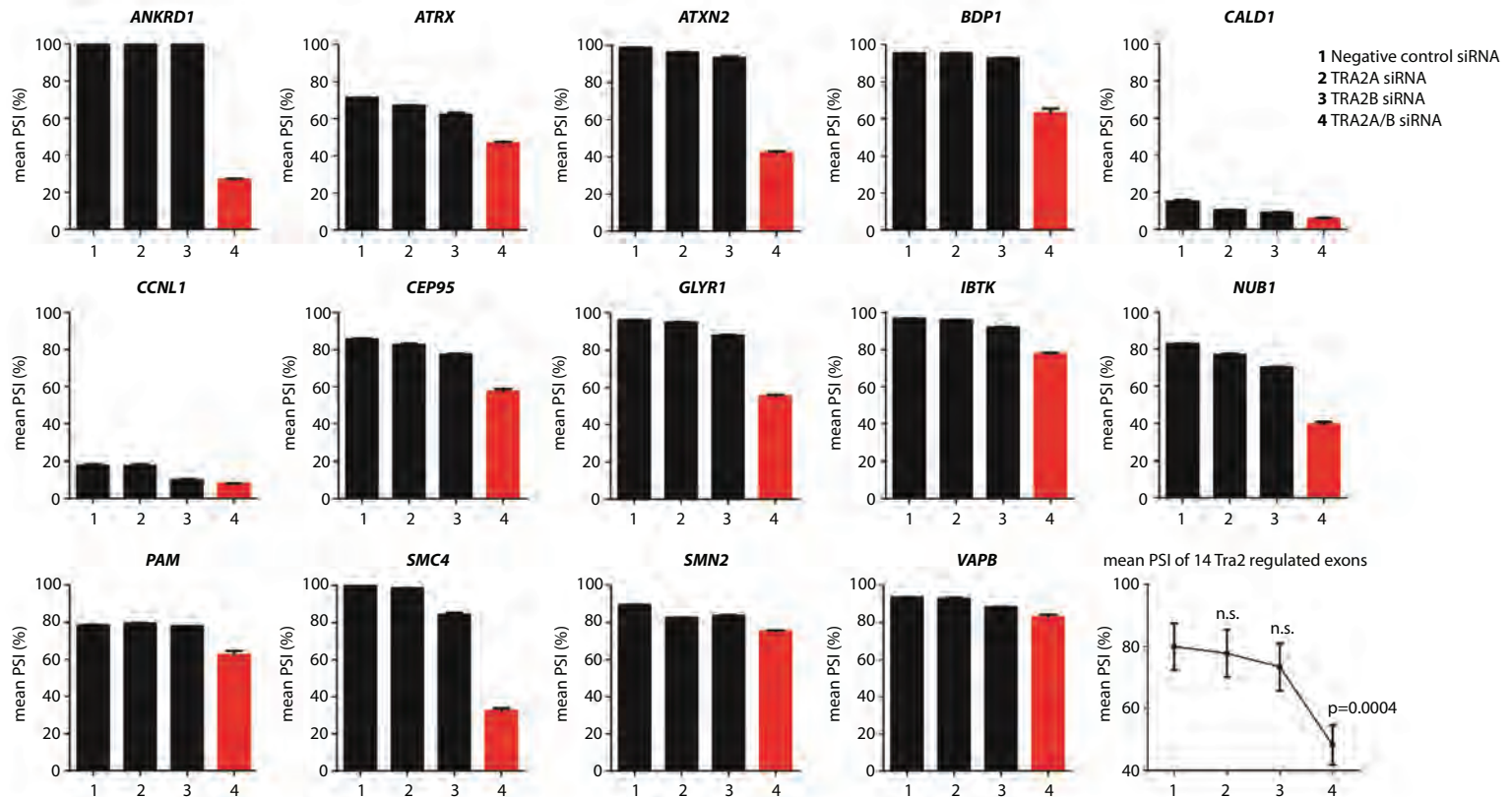
Supplementary Figure 2. Genomic landscape of Tra2 β protein binding in MDA-MB-231 cells.

(a) Autoradiograph showing ³²P-labelled RNA cross-linked to endogenous Tra2 β from MDA-MB-231 cells during an iCLIP experiment. The area highlighted in red was excised for further analysis.

(b) The top 10 most frequently occurring pentamers recovered by iCLIP analysis in MDA-MB-231 cells (GAA triplets are highlighted in red).

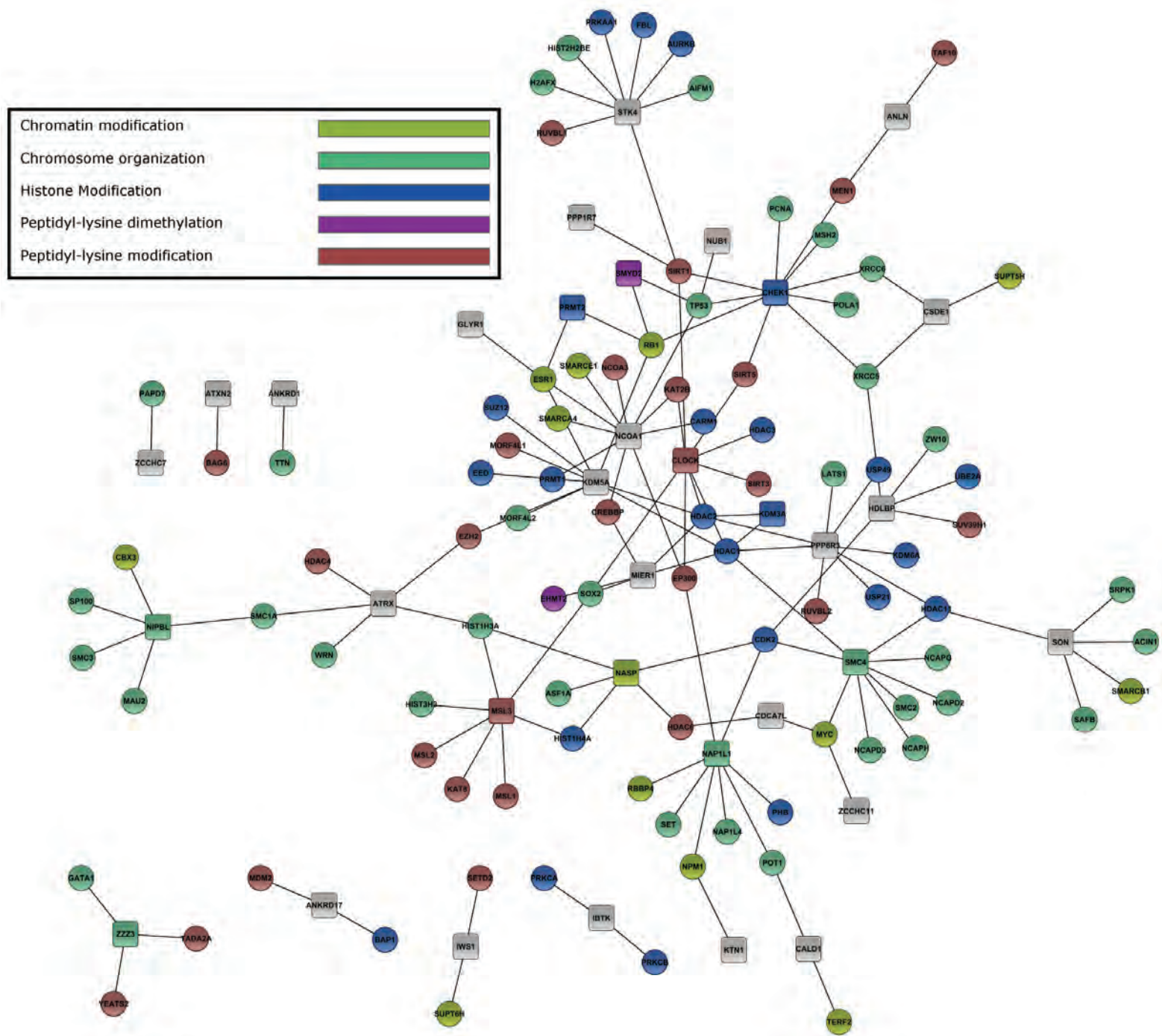
(c) Genomic distribution of Tra2 β binding sites. Total cross-links from triplicate iCLIP experiments (top panel) and relative enrichment of cross-links within each genomic region (number of cross-links relative to the size of each genomic region) (bottom panel).

Supplementary Figure 3.



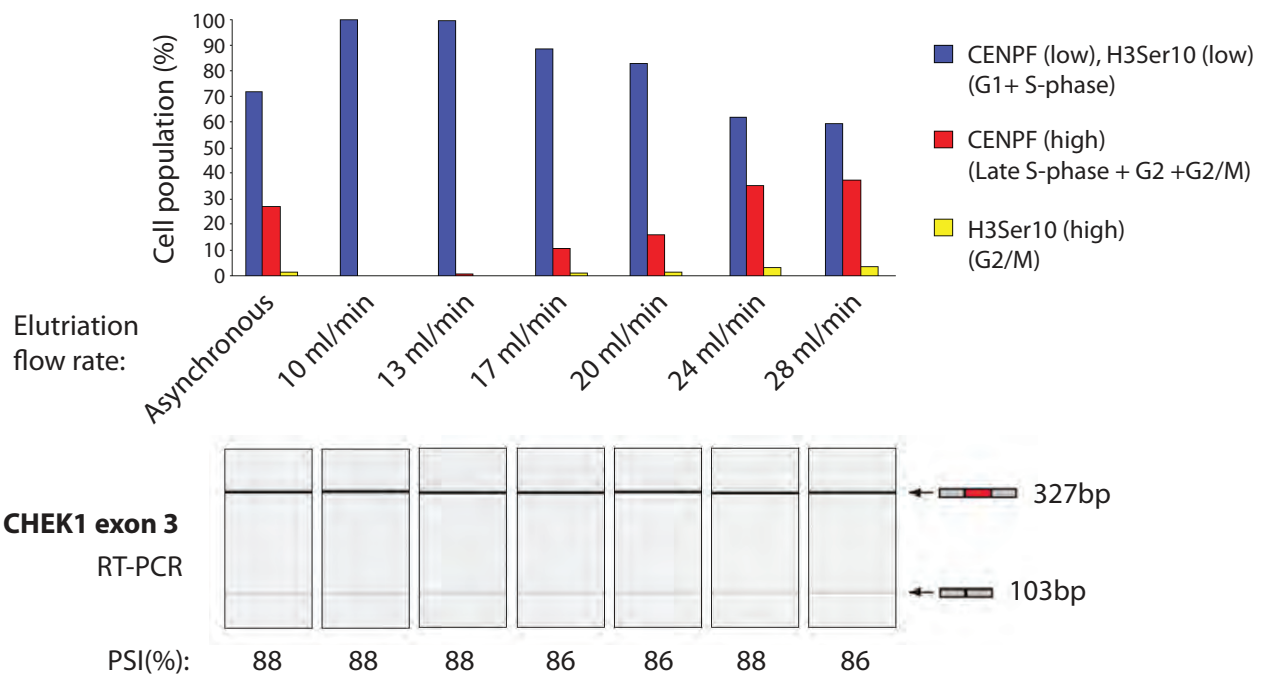
Supplementary Figure 3. RT-PCR validation of 14 Tra2-regulated exons in response to single depletion of Tra2 α or Tra2 β compared to joint depletion of both Tra2 α and Tra2 β . Splicing inclusion of 14 Tra2 β regulated exons was measured after single depletion of either Tra2 α or Tra2 β and following joint depletion of both Tra2 α and Tra2 β simultaneously. PSI levels were measured by RT-PCR using primers in flanking exons in three biological replicates. The mean PSI of all tested Tra2 β target exons was not significantly different from the control after single depletion of either Tra2 α or Tra2 β protein alone, but was highly significant after joint depletion of Tra2 α and Tra2 β , suggesting robust identification of Tra2-regulated exons would require joint Tra2 α and Tra2 β depletion prior to RNA-seq analysis.

Supplementary Figure 4.



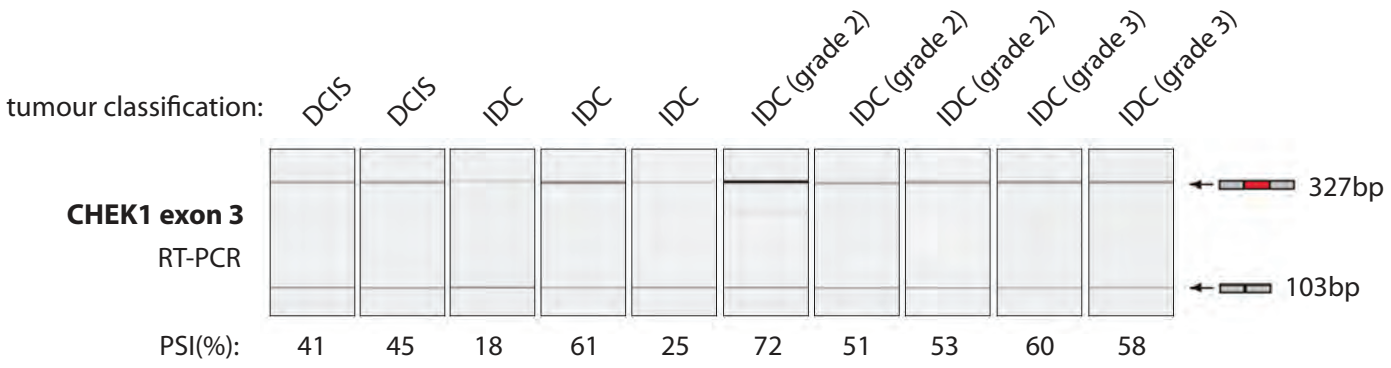
Supplementary Figure 4. Network analysis of Tra2-regulated exons. Network representation of genes whose splicing was functionally responsive to endogenous Tra2 protein (square nodes) and their interactions with genes/proteins annotated to the five enriched GO biological processes (circular nodes). Protein nodes are coloured by GO annotation as downloaded from QuickGO on 27th March 2014 (<http://www.ebi.ac.uk/QuickGO/>), and edges between nodes represent any type of functional interaction between the genes/proteins present in version 110 of the BioGRID database (<http://thebiogrid.org/>). Where a protein was annotated to more than one of the terms, the more specific child term was chosen for colouring. However, it should be noted that many of the genes in the network are annotated to multiple enriched terms.

Supplementary Figure 5.



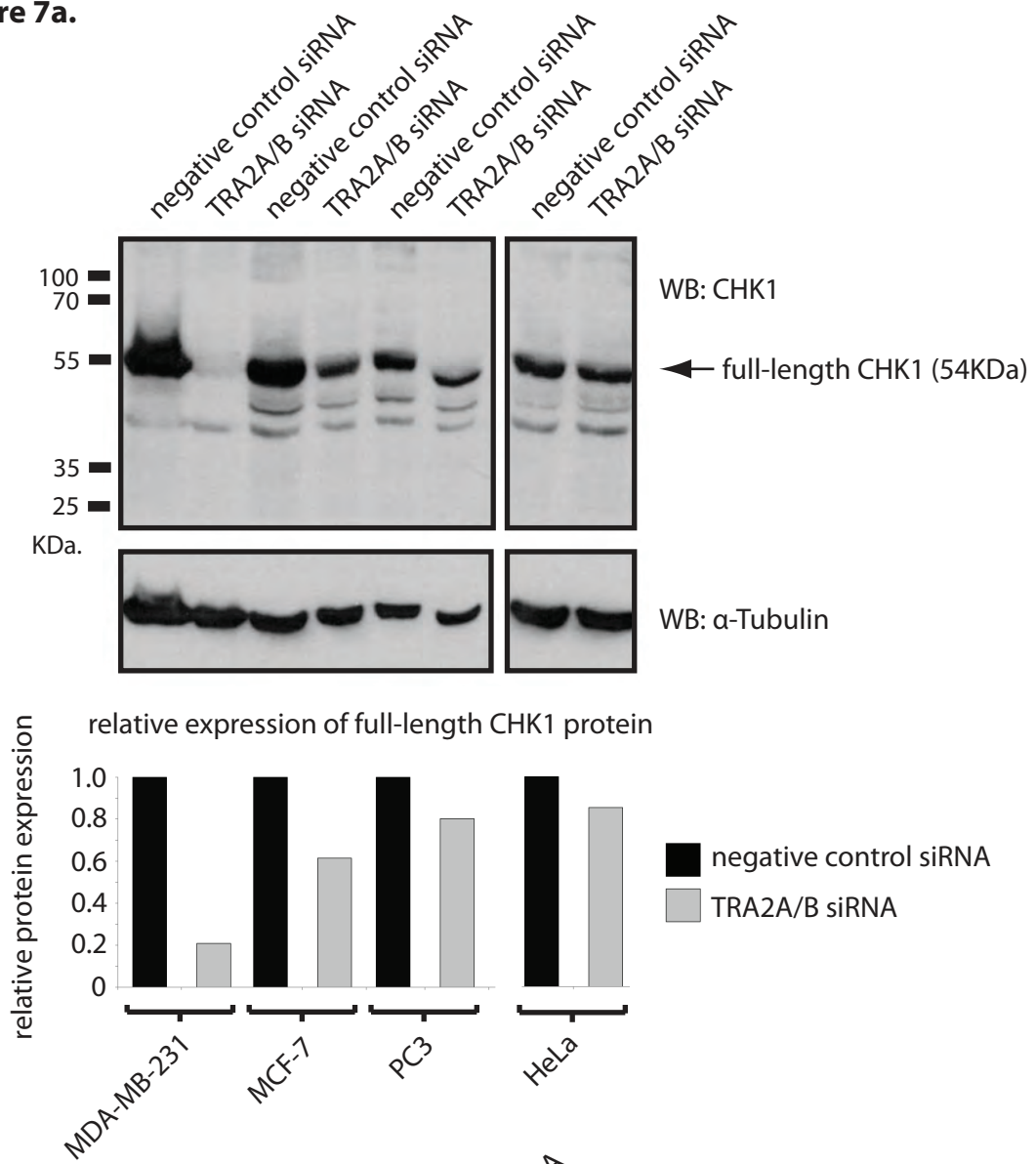
Supplementary Figure 5. (top): Bar chart showing asynchronous KG1 cells and cells from each elutriation flow rate fraction assessed for CENPF expression and phospho-histone H3Ser10 (H3Ser10) by immunofluorescence. For each marker, cells were scored as CENPF high or low and H3Ser10 high or low and the percentage of cells with each pattern plotted. Early fractions are negative for both markers consistent with G1 enrichment. Late S, G2, G2/M -associated CENPF staining appeared in an increasing number of cells from fractions 17-28 ml/min. (Bottom) Capillary gel electrophoretogram showing CHEK1 exon 3 splicing profile in different cell cycle fractions.

Supplementary Figure 6.

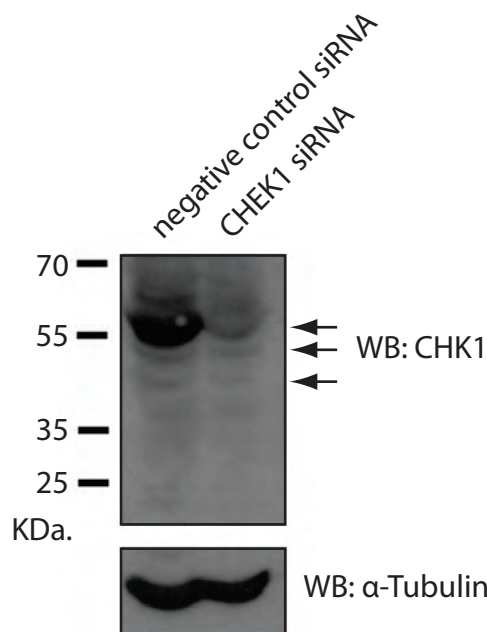


Supplementary Figure 6. CHEK1 exon 3 splicing profile in RNA purified from biopsies of different human breast cancers.

Supplementary Figure 7a.



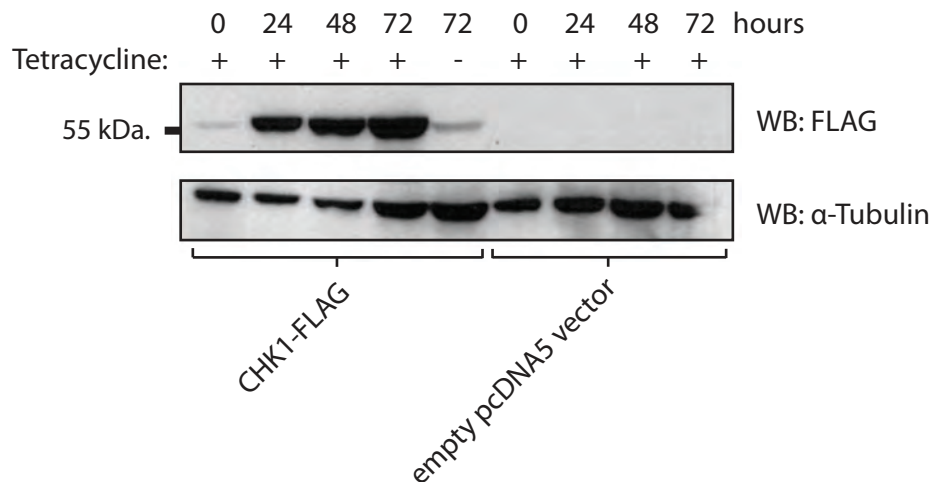
Supplementary Figure 7b.



Supplementary Figure 7. (a) Detection of CHK1 protein expression levels by Western blotting (upper panel); α -Tubulin protein expression levels in the same samples by Western blotting (middle panel); and the quantified relative levels of full-length CHK1 protein expression (54kDa band) in cells transfected with negative control siRNA compared to cells transfected with TRA2A/B siRNAs. The cell lines analysed were MDA-MB-231, MCF7, PC3 and HeLa.

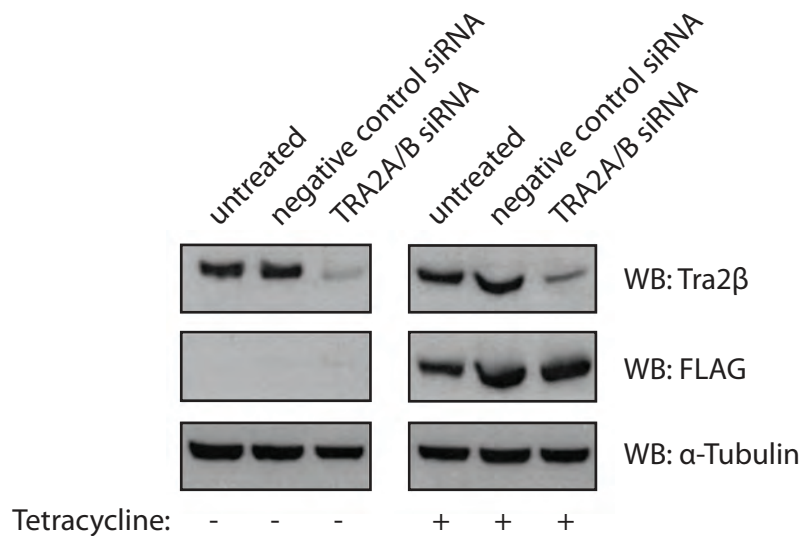
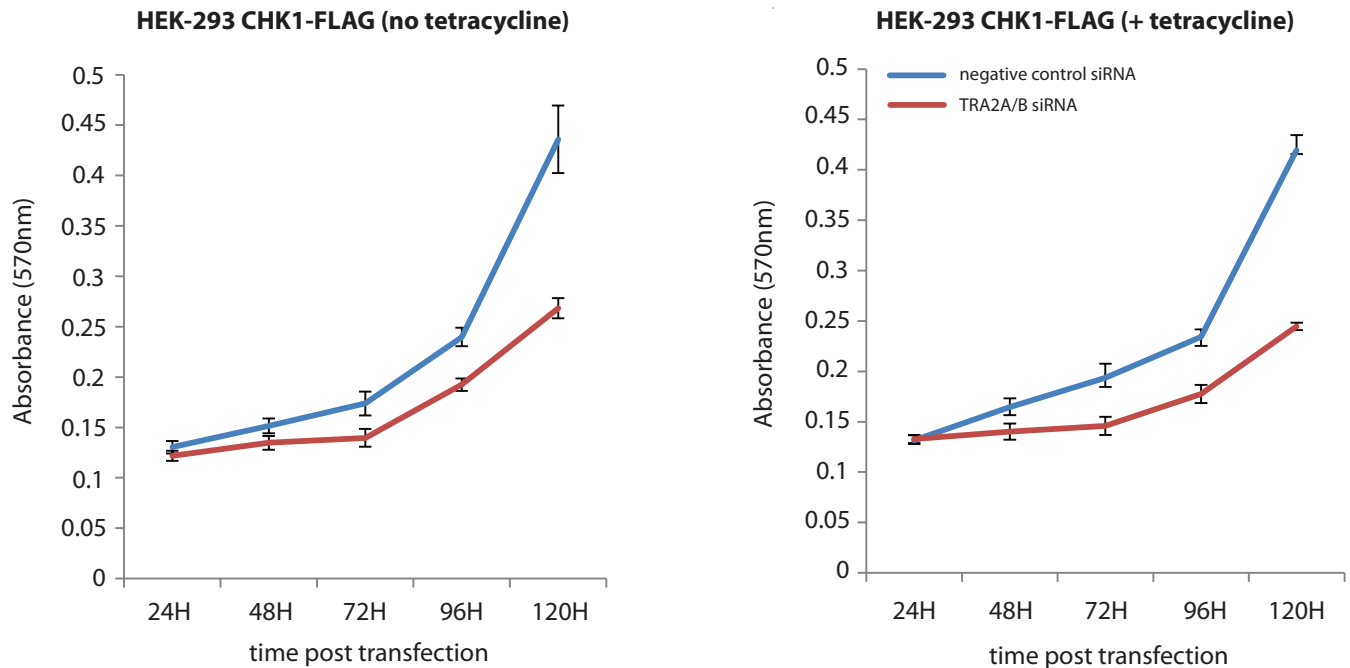
(b) Detection of protein isoforms in MDA-MB-231 cells after probing for CHK1 using the Proteintech antibody 10362-1-AP, and using a long period of chemiluminescent exposure to help detect less abundant proteins. α -tubulin is used as a loading control.

Supplementary Figure 8.



Supplementary Figure 8. Expression of FLAG-tagged CHK1 protein in response to tetracycline induction in a stable FLP-In HEK-293 cell line, compared with a control stable cell line made with empty vector. In each case a time-course of induction is shown after tetracycline addition with α -tubulin detected in parallel as a loading control.

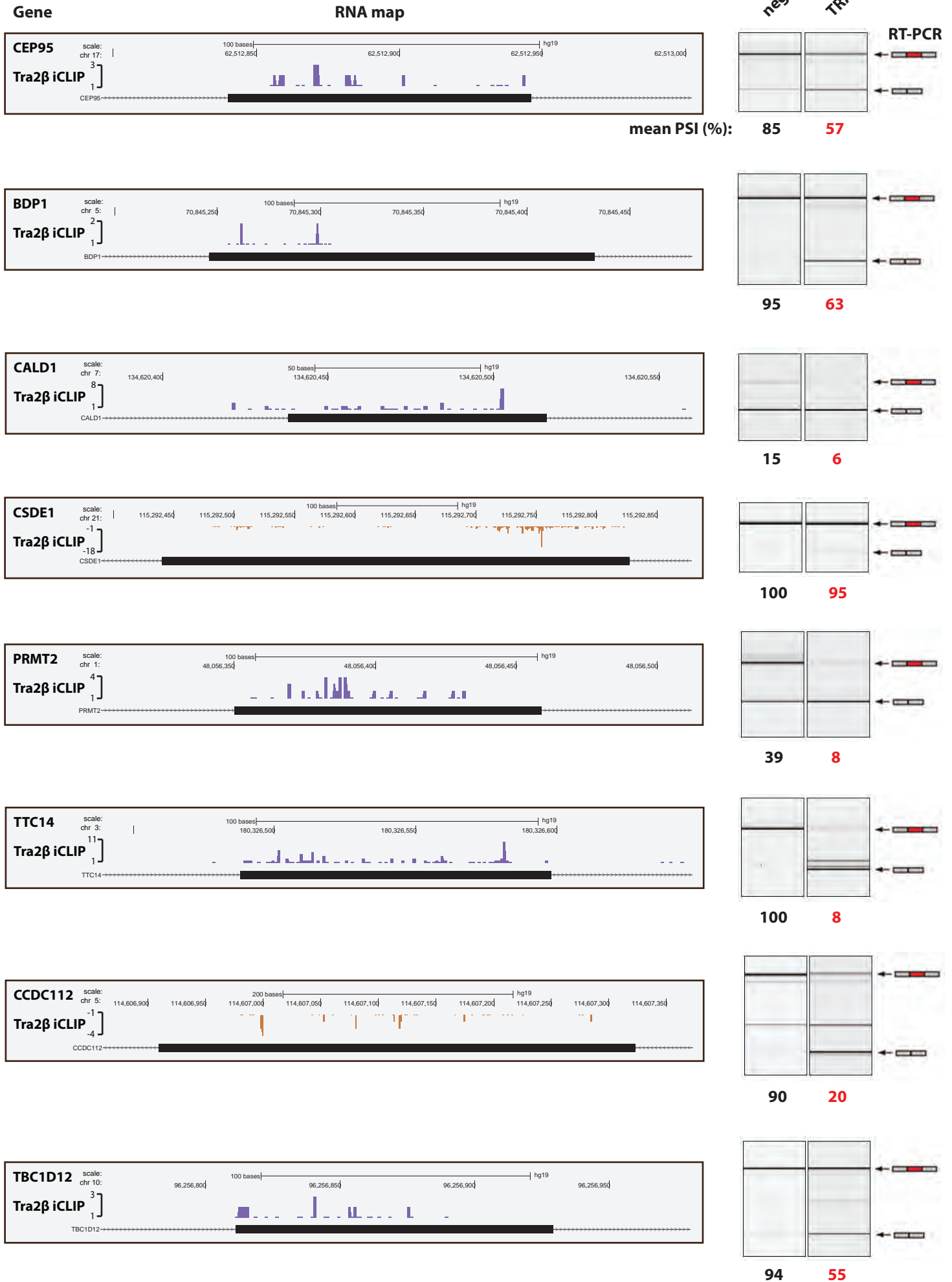
Supplementary Figure 9.

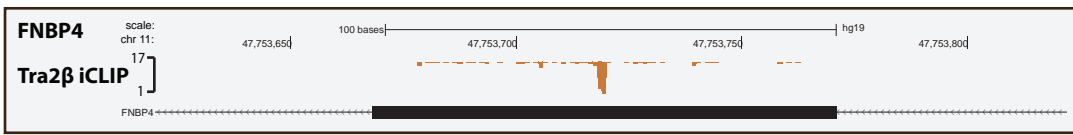


Supplementary Figure 9. Top: Graphs showing cell viability of the stable FLP-In T-REX cells grown without (left graph) or with tetracycline (right graph), following treatment with a control siRNA, or joint siRNA treatment to deplete Tra2 α and Tra2 β . FLP-In T-REX cell clones were used which stably express either CHK1-FLAG or were created with empty pCDNA5 vector. Underneath: panels showing Western blots from each stable cell line of FLP-In T-REX cells (left hand side, control cells; right hand side, cells expressing CHK1-FLAG) after different siRNA treatments, to show efficiency of Tra2 β depletion and expression of CHK1-FLAG protein.

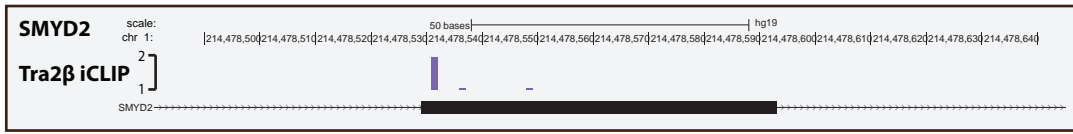
Supplementary Figure 10 - RNA maps of TRA2 responsive exons

negative control siRNA
TRA2A/B siRNA

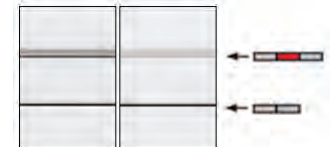
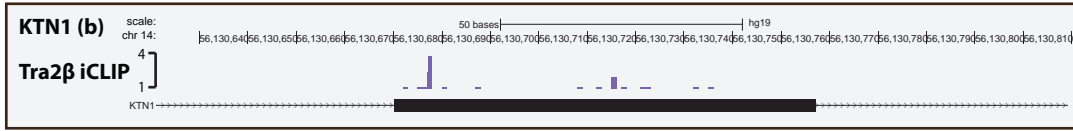




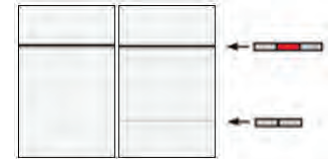
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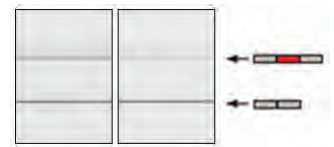
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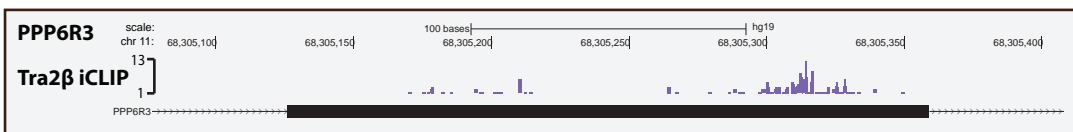
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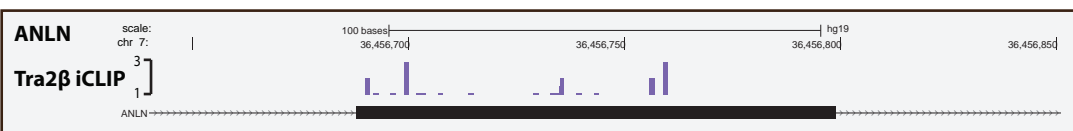
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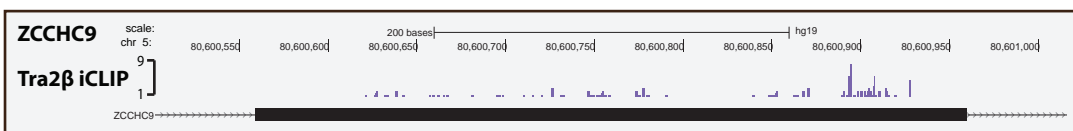
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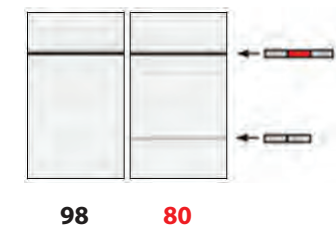
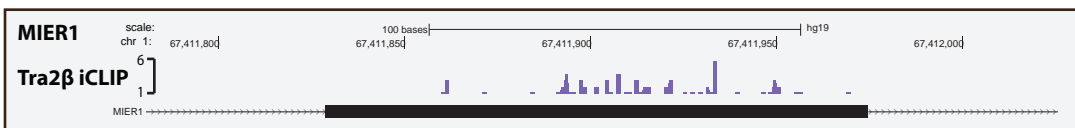
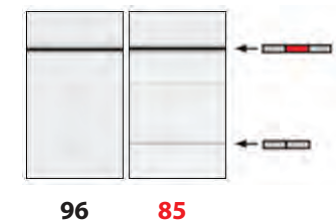
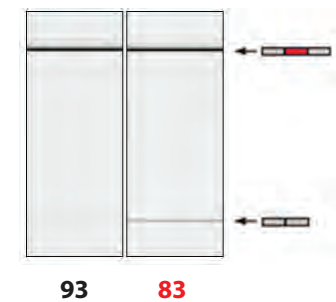
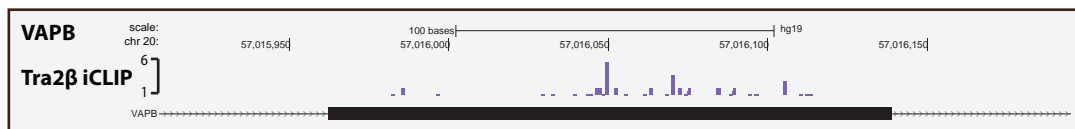
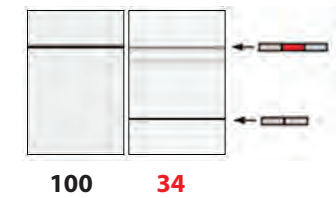
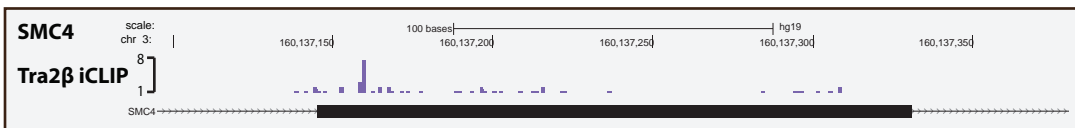
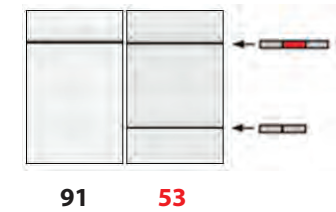
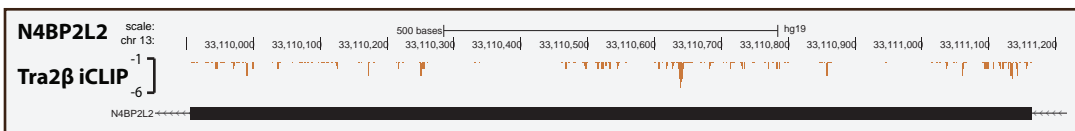
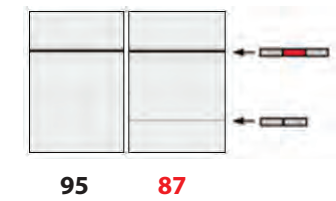
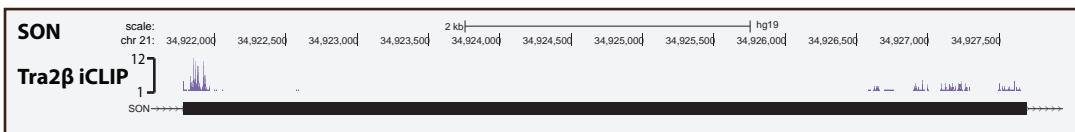
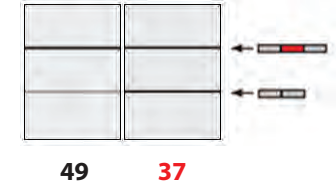
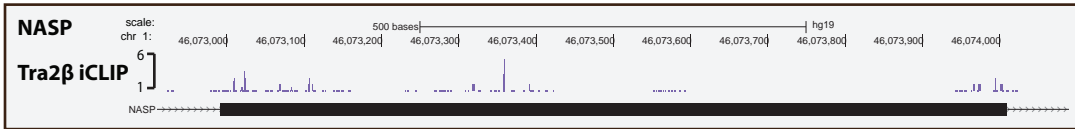
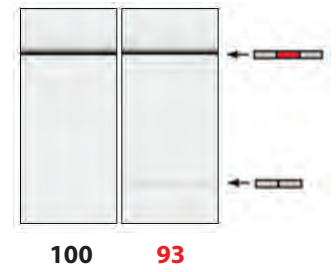
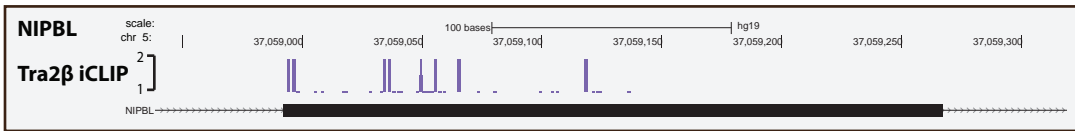
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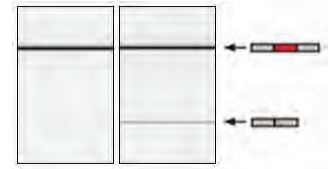
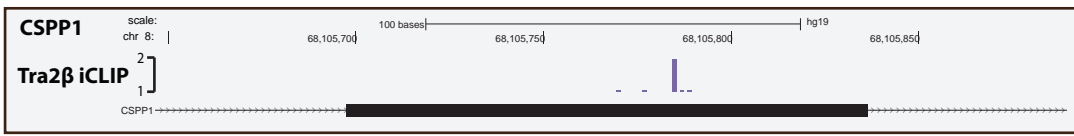


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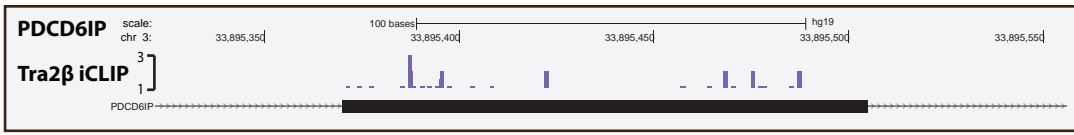


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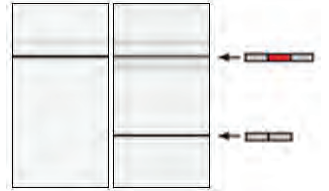




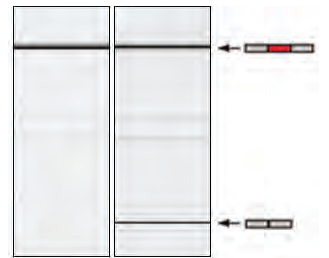
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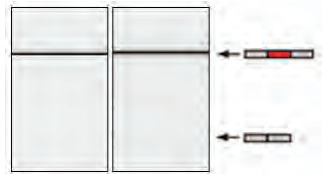
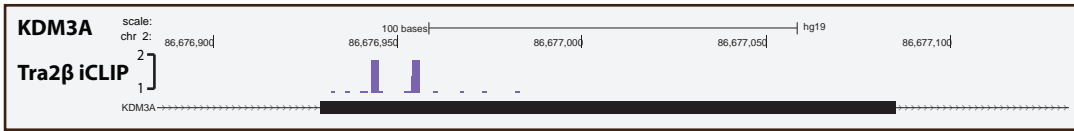
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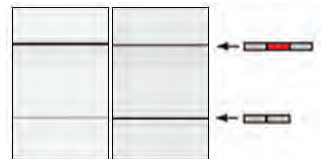
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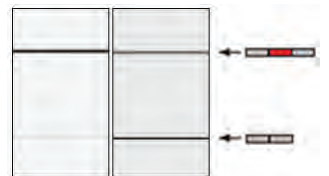
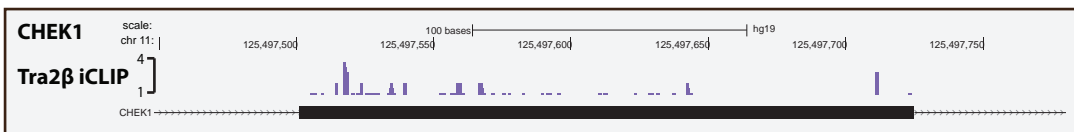
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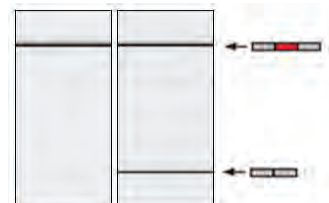
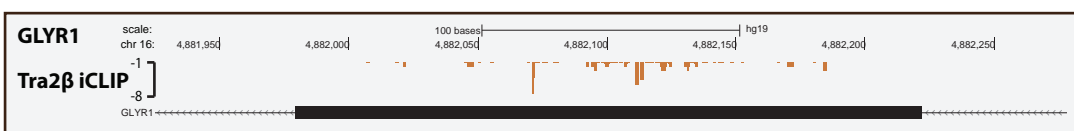
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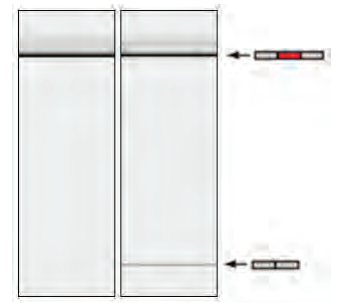
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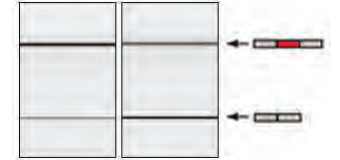
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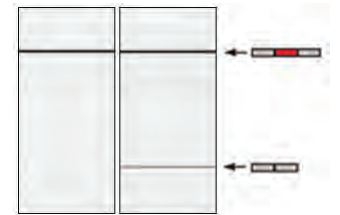
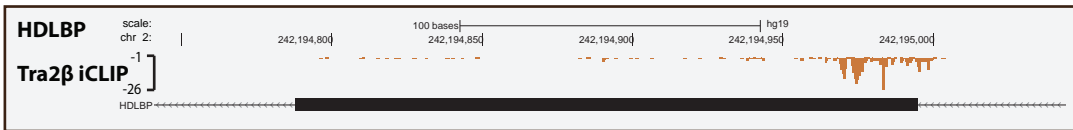
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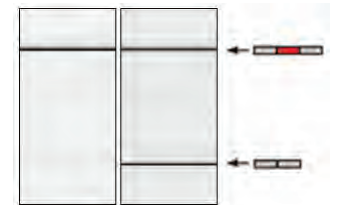
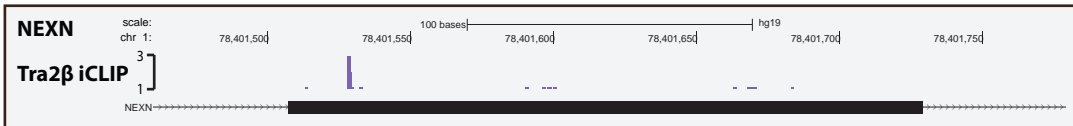
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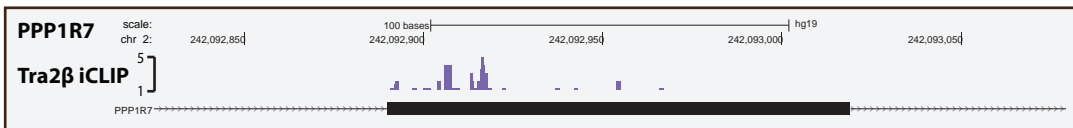
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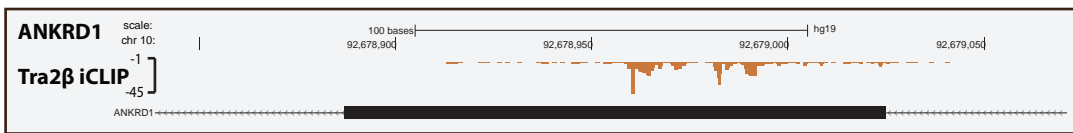
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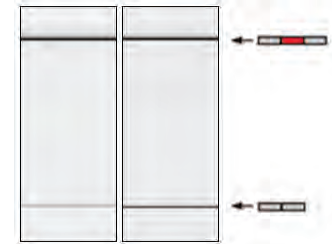
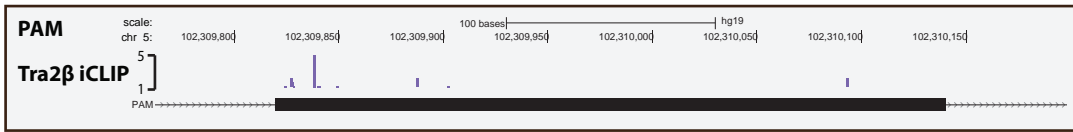
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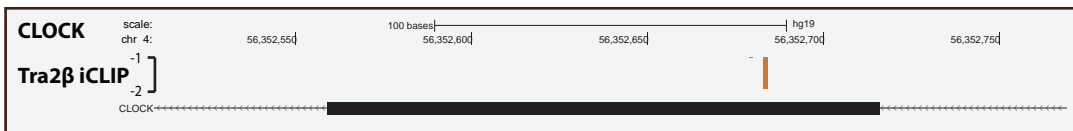
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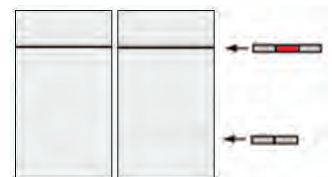
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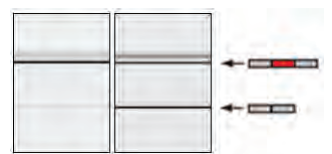
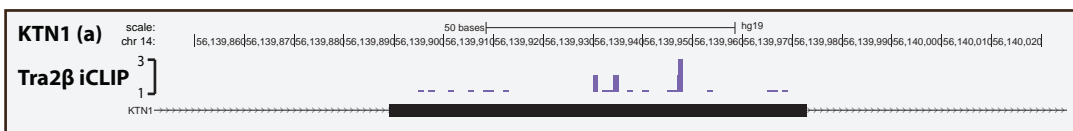
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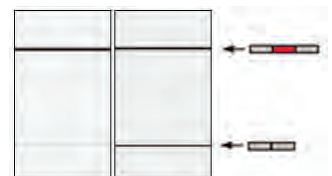
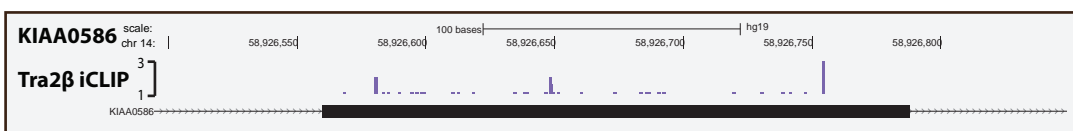
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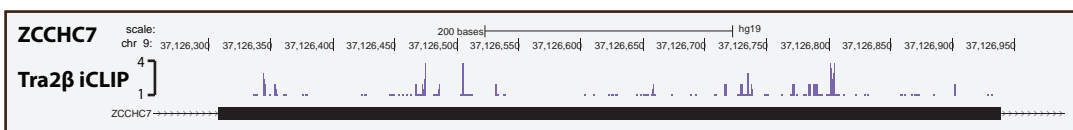
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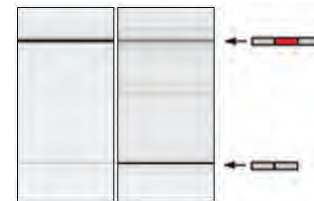
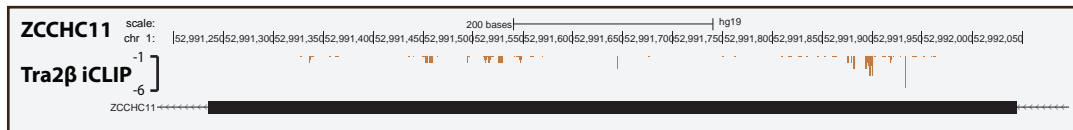
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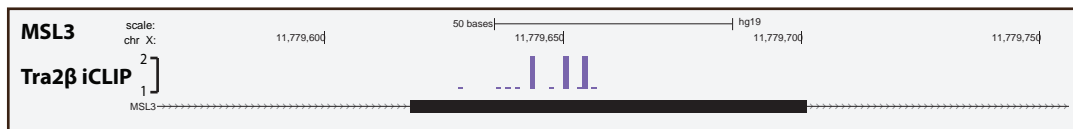
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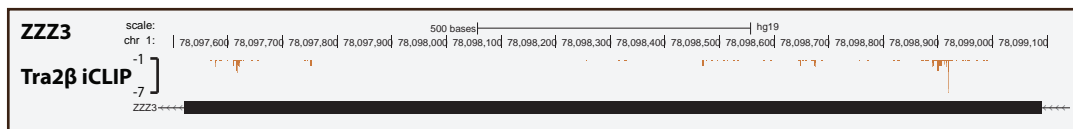
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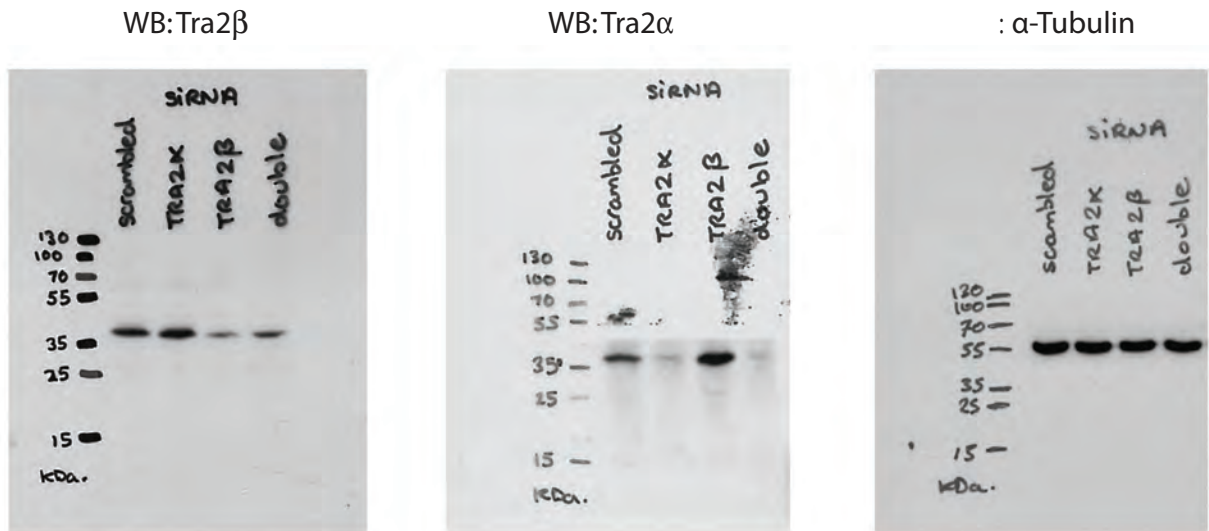


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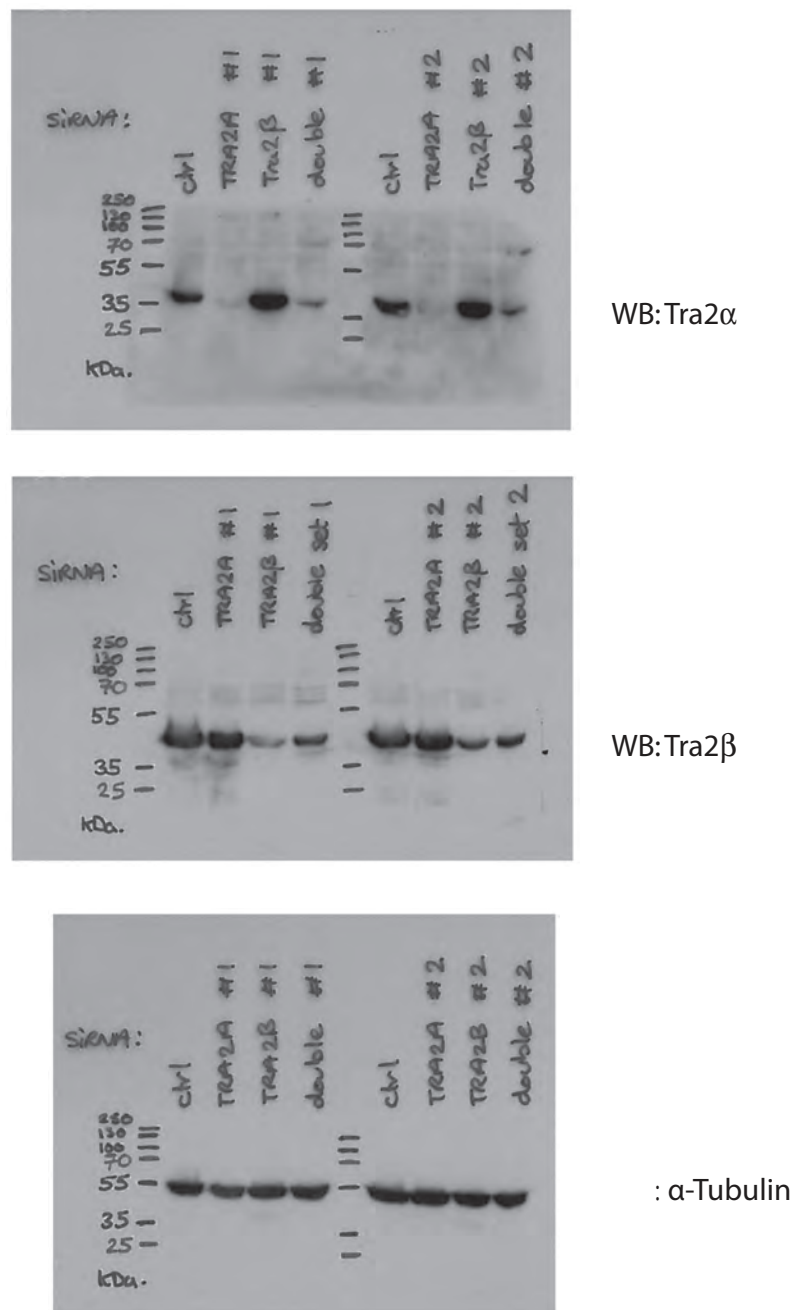
Supplementary Figure 10. iCLIP maps and RT-PCR validation of 53 Tra2-regulated exons that changed splicing profile in response to joint Tra2 depletion. Images of exons that responded to joint Tra2 protein depletion are shown as screenshots from the UCSC genome browser 33. Joint Tra2α/Tra2β responsive exons were identified using combined iCLIP and RNA-seq analysis and splicing was monitored by RT-PCR using primers within flanking exons. PSI (%) levels were measured in MDA-MB-231 cells transfected with a control siRNA or after joint transfection with siRNAs specific to TRA2A and TRA2B in three biological replicates.

Uncropped Western blots and gels

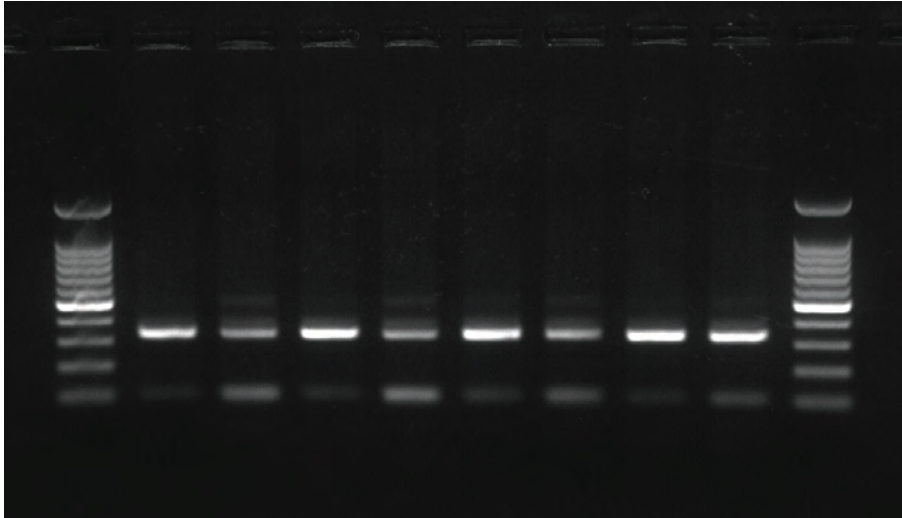
Supplementary Figure 11 - uncropped Western blots corresponding to Figure 1a



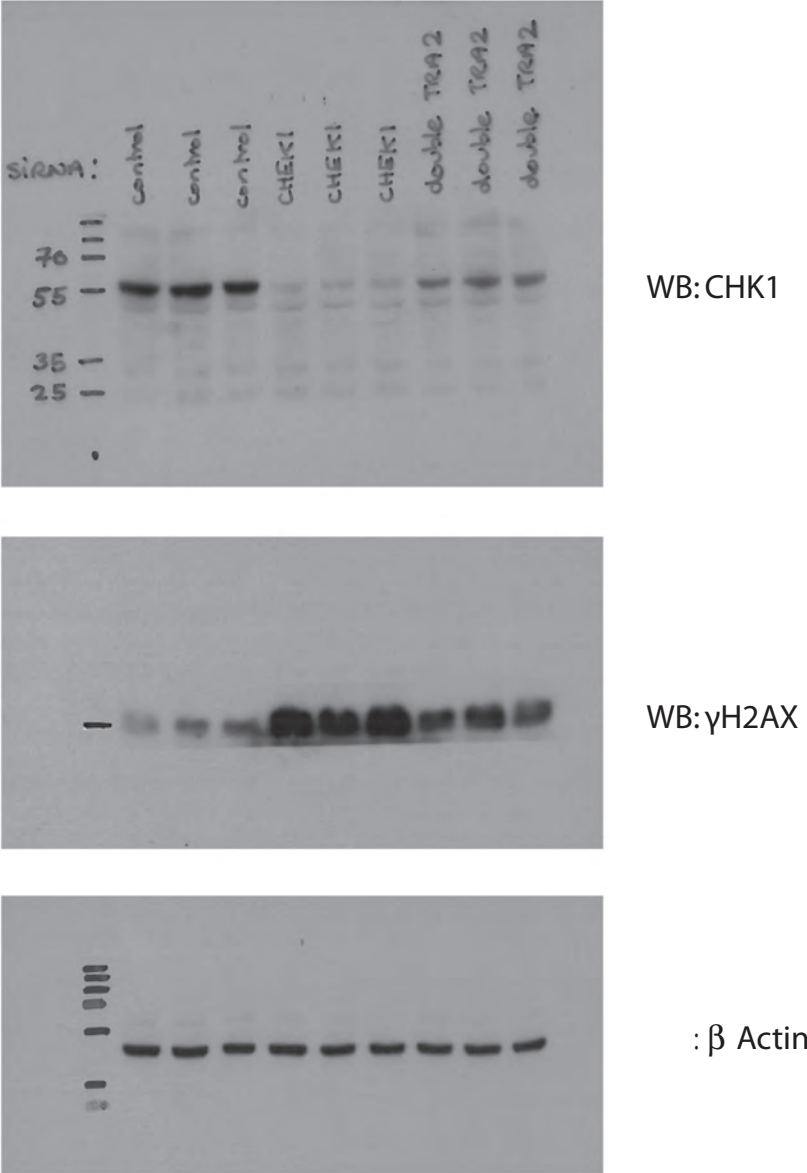
Supplementary Figure 12 - uncropped Western blots corresponding to Supplementary Figure 1



Supplementary Figure 13 - uncropped gel corresponding to Figure 6b

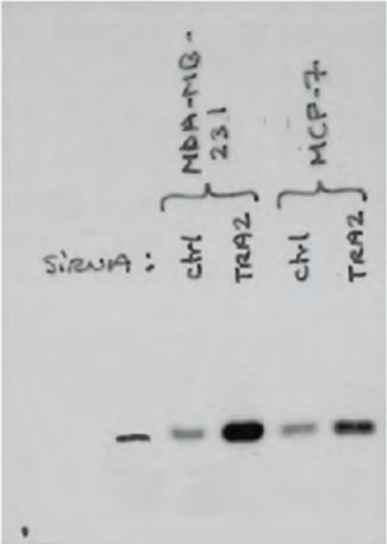


Supplementary Figure 14 - uncropped Western blots corresponding to Figure 8a

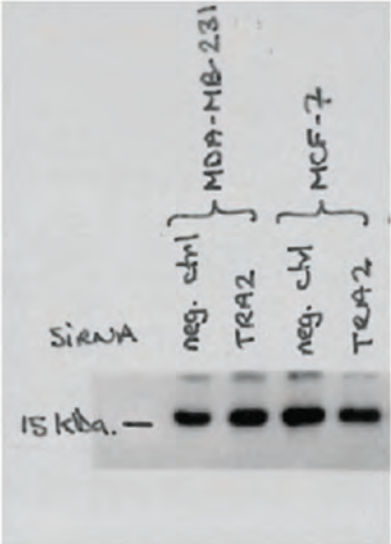


Supplementary Figure 15 - uncropped Western blots corresponding to Figure 8b

WB: γ H2AX



WB: total H2AX



: α -Tubulin

