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Supplemental information

**The global Protein-RNA interaction map of ESRP1
defines a post-transcriptional program
that is essential for epithelial cell function**

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Supplementary Figure Legends

Figure S1. Epidermis from *Esrp1*^{FLAG/FLAG} mice used for eCLIP (related to STAR Methods).

A. Ethidium Bromide stained agarose gel of (A)val, (H)incll or uncut PCR product from *Fgfr2* mutually exclusive exon in epidermis from *Esrp1*^{FLAG/FLAG} and *Esrp1*^{WT/WT} mice B. CLIP Optimizations for the Epidermis (Left) Autoradiogram of crosslinked and non-crosslinked FLAG ESRP1 IP showing RNA: Protein products following limited digestion with RNase I at 0, 5, 20 and 40U. (Right) Western Blot of lysates of FLAG ESRP1 and non-FLAG tagged ESRP1 showing input and immunoprecipitated proteins. Hatched box indicates portion of the membrane extracted for ESRP1:RNA complexes.

Figure S2. Analysis of ESRP1 regulation of alternative splicing by MAJIQ and rMATs (related to Figure 2).

A. Number and type of alternatively spliced events identified by MAJIQ between control and experimental epidermis. Putative events only have one junction identified, and could not be attributed to any of the other classes B. Correlation plot of Δ PSI estimated by MAJIQ of 23 skipped exon events validated by RT-PCR, shown in on graph are Pearson Correlation *r* and *p*-values. Green dots represent splicing events suppressed by ESRP1 and red dots represent splicing events enhanced by ESRP1. C. Correlation plot of for Δ PSI skipped exon estimated by rMATs and Δ PSI binary skipped exon events estimated by MAJIQ. E. Voila plots showing examples of the detection of alternative last exon events MAJIQ for *Gpatch2*. Hatched lines indicate splicing event reported in the database but not detected in the RNA-seq, solid lines indicate splicing event detected in the RNA-seq. Red lines show splicing to the ALE exon 9, blue lines show splicing to ALE exon 10 and green lines indicate splicing to ALE exon 16.

Figure S3. Complex regulation of alternative splicing by ESRP1 (related to Figure 3).

Genome browser views showing ESRP1 binding (CLIP Seq) and transcript levels (RNA-seq) of for *Cd44*(A), *Far1* (B), and *Fgfr2* (C). A. Alternative splicing of *Cd44* is regulated by ESRP1. Voila plots show differential exon inclusion between Control (CON) and Experimental (cDKO) conditions for *Cd44*, showing ESRP1 enhances inclusion of isoform variable exons 1-10. CLIP-seq densities show CLIP and size matched input, and transcript levels are from RNA sequence in epidermis ablated of *Esrps* and control epidermis. Individual Crosslink sites identified by PureCLIP are shown for three replicate pairs at top of the browser

views, Peaks called by piranha are indicated above the browser view in red. Conservation scores showing Placental mammal conservation by PhyloP at the base. B. ESRP1 binding and regulation of *Far1*. Genome browser views showing ESRP1 binding (CLIP Seq) and transcript levels (RNA-seq) of for *Far1*. CLIP-seq densities show CLIP and size matched input, and transcript levels are from RNA sequence in epidermis ablated of *Esrps* and control epidermis. Crosslink sites identified by PureCLIP are shown for three replicate pairs. Conservation scores showing Placental mammal conservation by PhyloP at the base. Multiple alignment of ~50 nucleotide region under ESRP1 peak for several vertebrate species shown. Highlighted in green are UGG motifs recognized by ESRP1. Voila plots of ESRP1 regulated mutually exclusive exon (MXE) splicing event in *Far1*. C. ESRP1 binding and regulation of *Fgfr2*. Genome browser views showing ESRP1 binding (CLIP Seq) and transcript levels (RNA-seq). CLIP-seq densities show CLIP and size matched input, and transcript levels are from RNA sequence in epidermis ablated of *Esrps* and control epidermis. Crosslink sites identified by PureCLIP are shown for three replicate pairs. Voila plots show differential exon inclusion between Control (Con) and Experimental (Exp) conditions for *Fgfr2*.

Figure S4. ESRP1 Enhanced Cassette Exon Events (related to Figure 3).

(Top) Ethidium bromide-stained agarose gel of restriction digest of FGFR2 RT-PCR products in HEK293T cells upon transfection with either mCherry (control), ESRP1 WT, ESRP1 RRM* (ESRP1 RRM Mutant), or untransfected cells. ESRP1 WT is able to promote splicing of FGFR2 IIIb exon as shown by appearance of *Ava*I digested product and reduction in *Hinc*II digested product compared to ESRP1 RRM* (Bottom) Browser views of ESRP1 enhanced splicing events for *Atp6v1c2*, *Ralgps*, *Slc37a2* and *Uap1* showing ESRP1 peaks located downstream of the regulated exon where it enhances inclusion of the exon. Genome browser views showing ESRP1 binding (CLIP Seq) and transcript levels (RNA-seq). CLIP-seq densities show CLIP and size matched input, and transcript levels are from RNA sequence in epidermis ablated of *Esrps* and control epidermis. Crosslink sites identified by PureCLIP are shown for three replicate pairs and peaks identified by Piranha are shown at top of the browser views. Ethidium bromide-stained agarose gel of RT-PCR products of ESRP1 regulated splicing event from 293T cells assessing requirement for ESRP1 binding RNA to regulate splicing event, Lane 1 – control mCherry, Lane 2 – ESRP1 WT, Lane 3 – ESRP1 RRM mutant.

Figure S5. ESRP1 Silenced Cassette Exon Events (related to Figure 3).

Browser views of ESRP1 enhanced splicing events for *Magi1*, *Flnb*, *Scrib*, and *Timm17b* showing ESRP1 peaks located in the upstream intron and/or within the regulated exon where it suppresses inclusion of the exon. Genome browser views showing ESRP1 binding (CLIP-Seq) and transcript levels (RNA-seq). CLIP-seq densities show CLIP and size matched input, and transcript levels are from RNA sequence in epidermis ablated of *Esrps* and control epidermis. Crosslink sites identified by PureCLIP are shown for three replicate pairs and peaks identified by Piranha are shown at top of the browser views.

Figure S6. Splicing RNA Maps for ESRP1 (related to Figure 3).

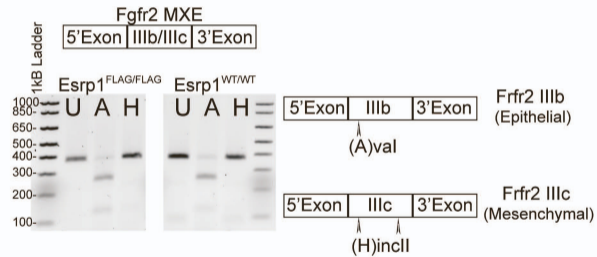
Splicing RNA Map of distribution of ESRP1 CLIP peaks on alternative 3' splice site (A3SS) (A) on alternative 5' splice site (A5SS) (B) and on mutually exclusive exons (MXE) (C). Solid blue line shows the ESRP1 CLIP densities over ESRP1 downregulated exons or A3SS, and dotted blue line shows the significance of the peak at each position. Solid red line shows the ESRP1 CLIP densities of ESRP1 upregulated exons or A3SS, with dotted red line indicating significance (p-value) of the binding at the position shown.

Figure S7. ESRP1 binding within the 3'UTR differentially affects mRNA and protein levels (related to Figure 6).

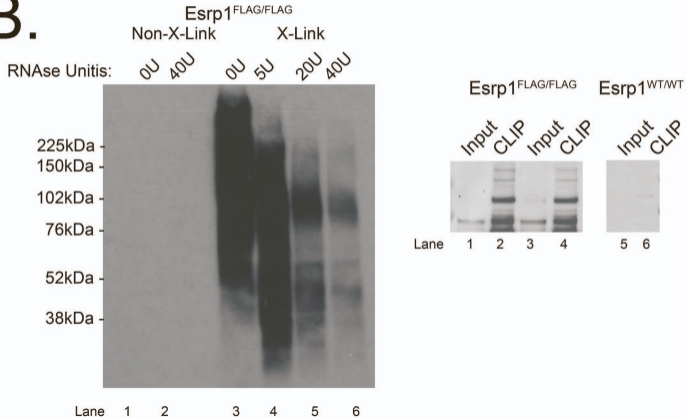
A. Dot plot of TPM values from epidermis ablated of *Esrps* and control epidermis. Genes shown except for *Esrp1* and *Gapdh* had ESRP1 peaks within the 3'UTR. Data are represented as mean +/- SD. In general, genes show no change in the mRNA levels. *Esrp1* knockout deletes exons 7-9 of the transcript deleting RRM1 and functional knockout confirmed previously (Beebe et al., 2015). B. Quantification of western blot of Py2T cell lines, *WT* and *Esrp1*^{-/-}, assessing protein levels of eCadherin, CLAUDIN4, CTNNB1 and CORTACTIN C. Data are represented as mean +/- SD. Luciferase reporter assay (pIS0) comparing effects of Nuc-ESRP1 (+CKLP) or Cyto-ESRP1 (-CKLP) on *Ctnnd1* UTR and *Gapdh* UTR, and *Cdh1* UTR, *Cldn4* UTR. Data are represented as mean +/- SD.

Supplement Figure 1

A.

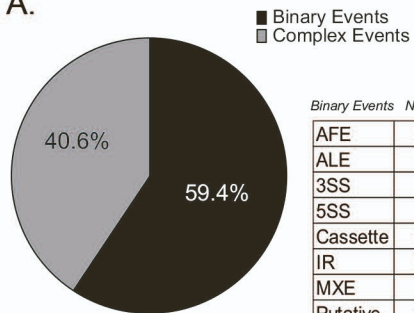


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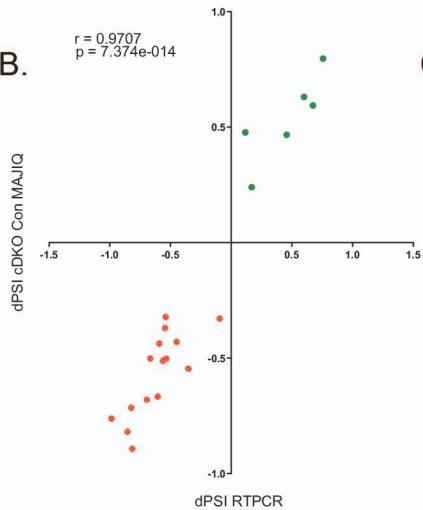


Supplementary Figure 2

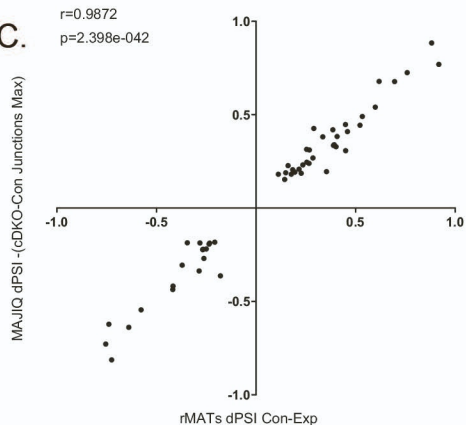
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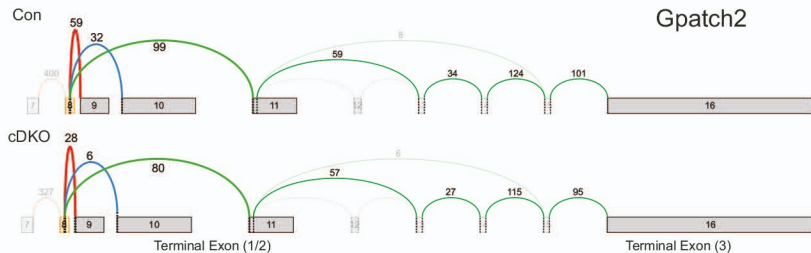
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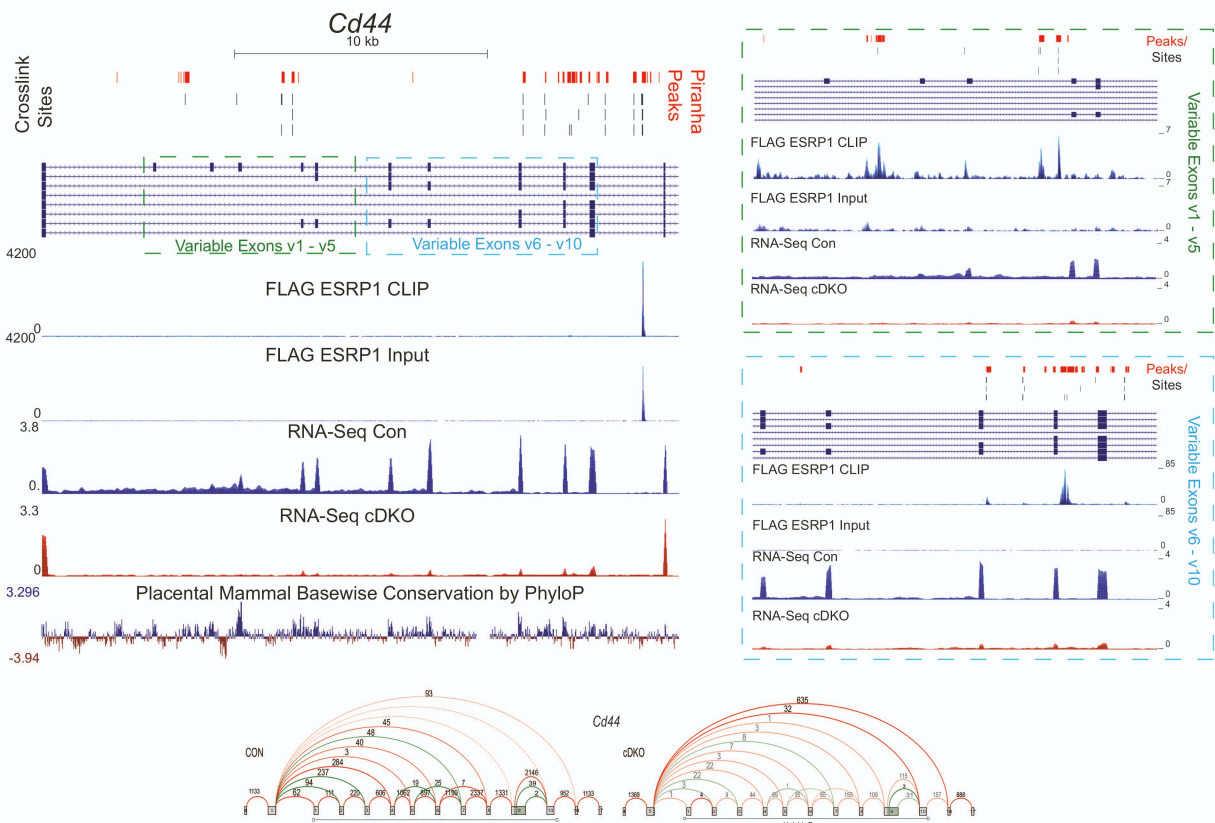
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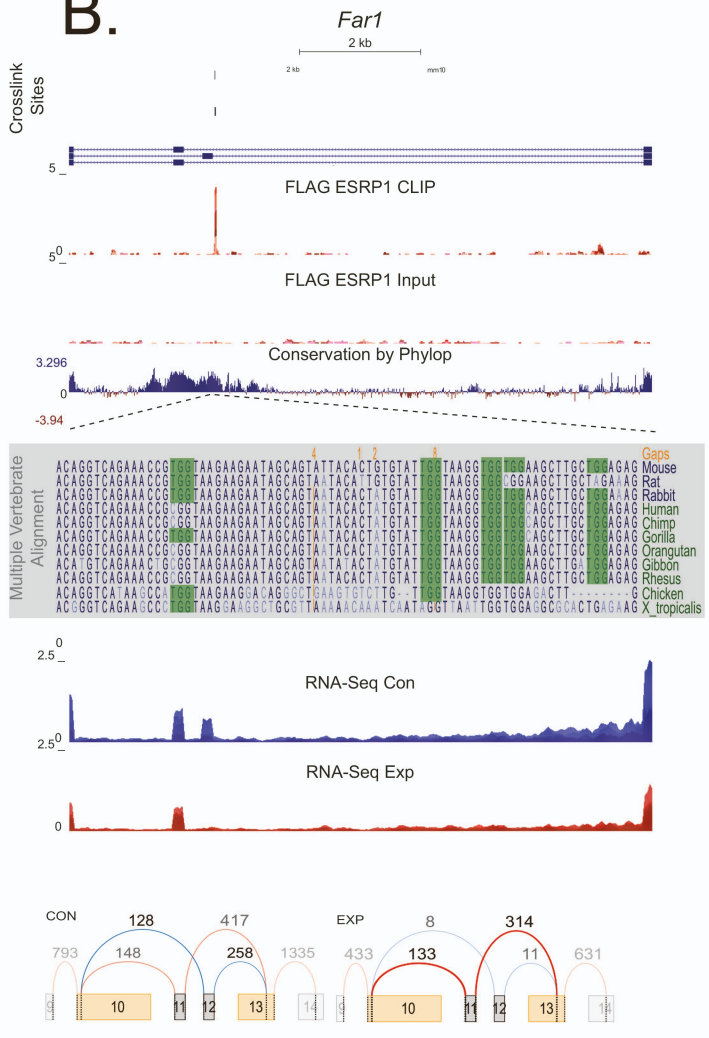
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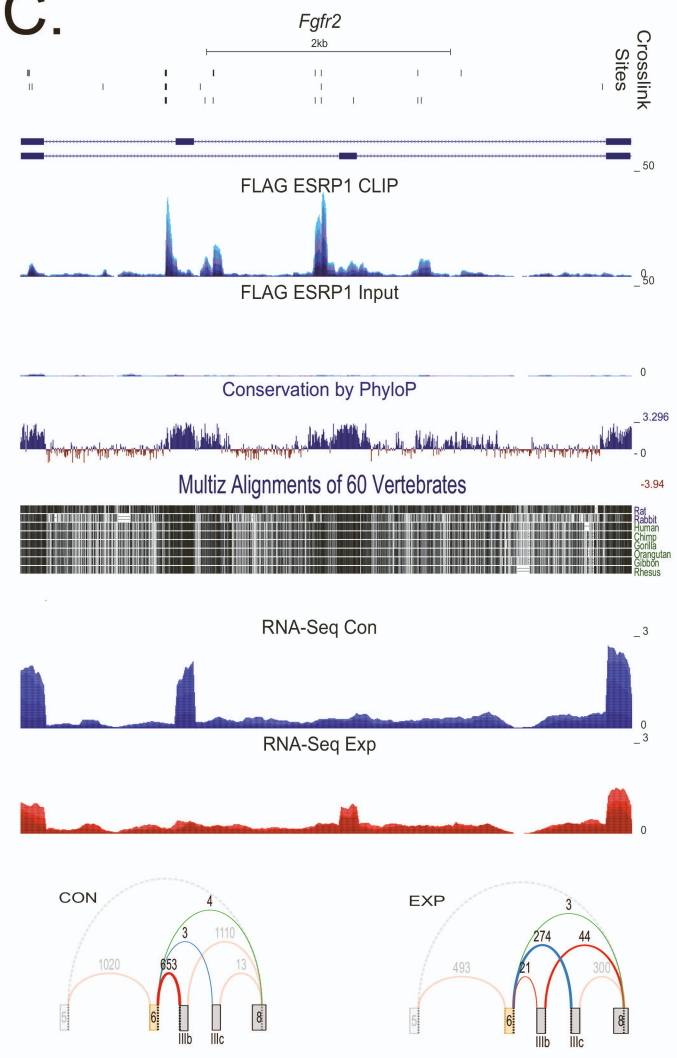
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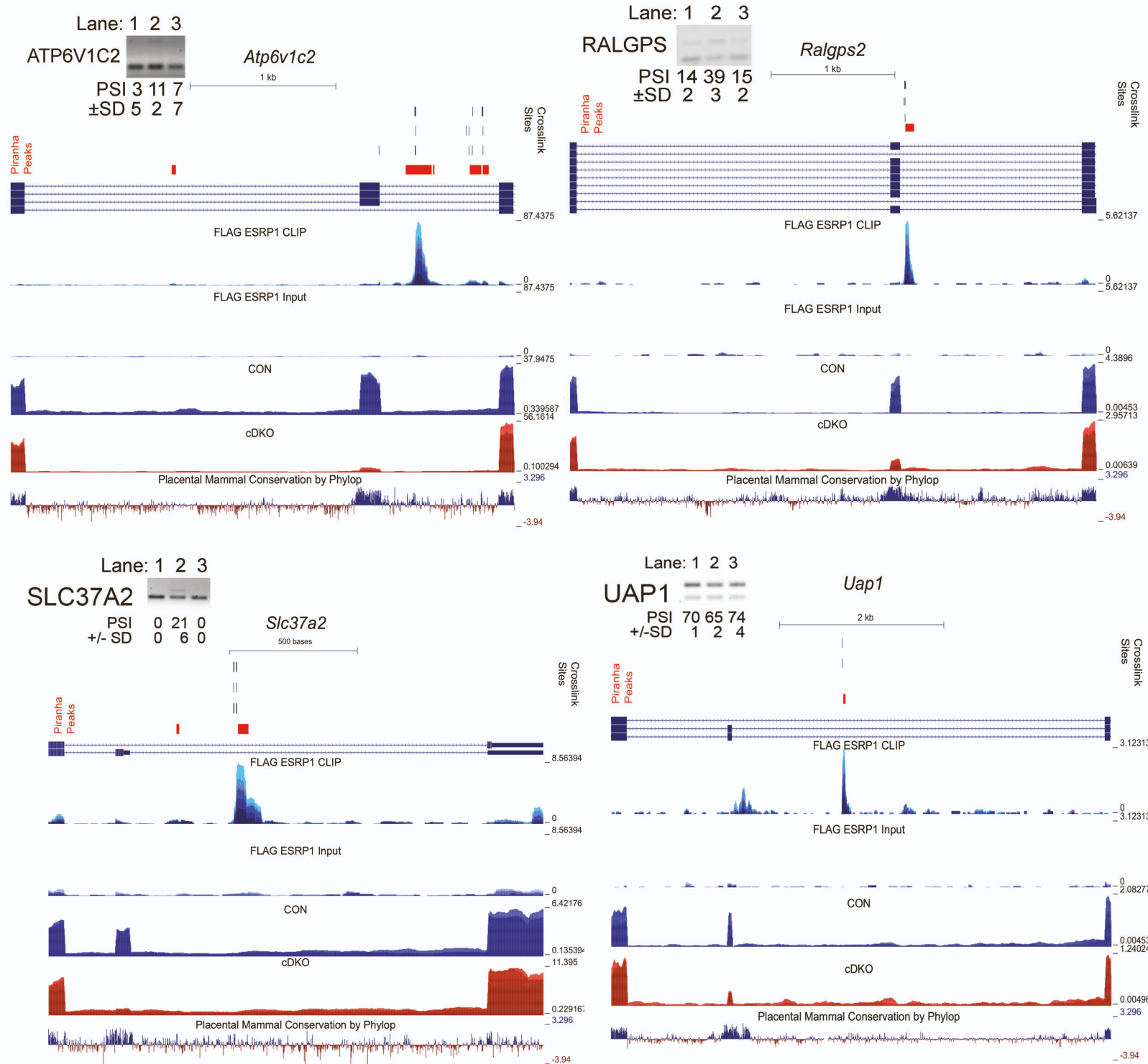


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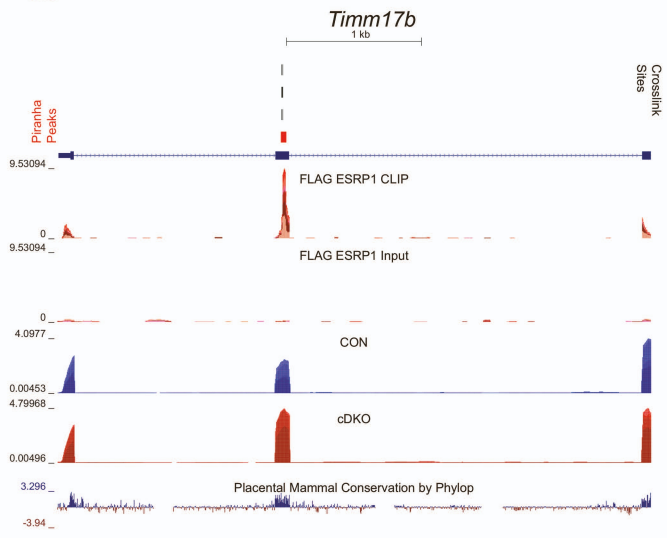
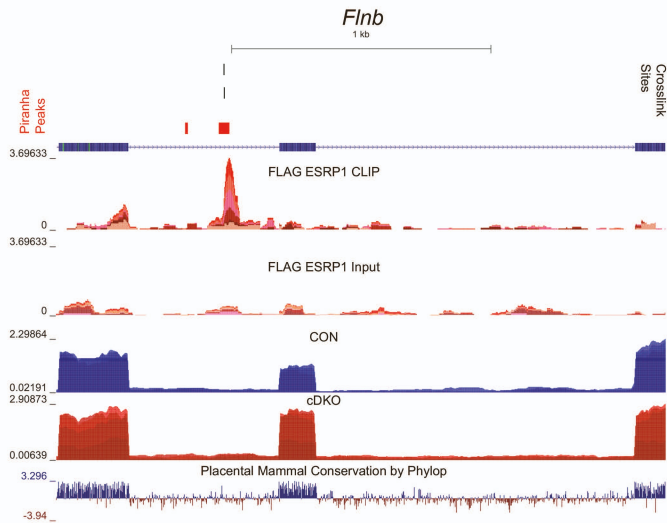
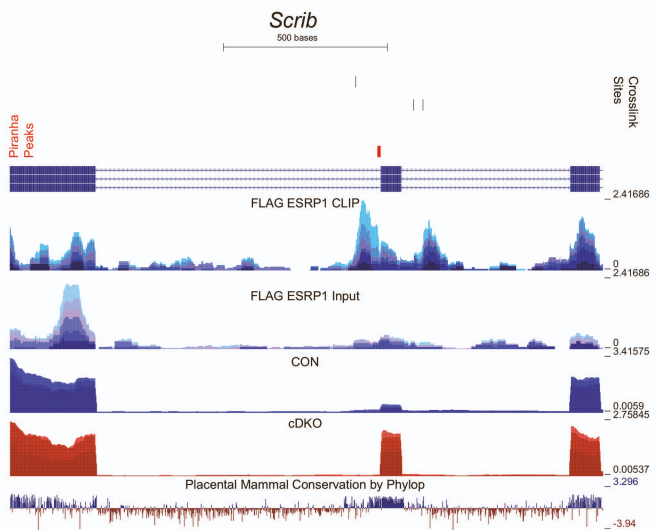
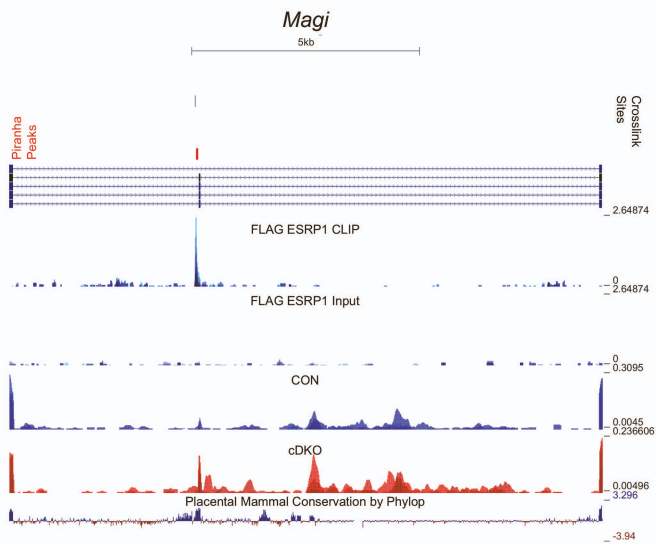
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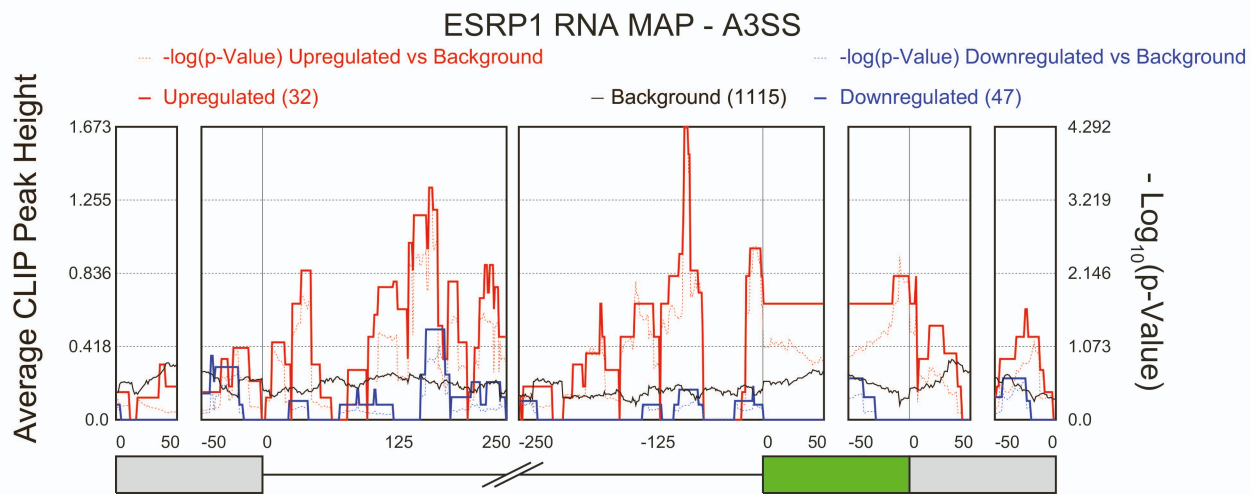


Supplementary Figure 4

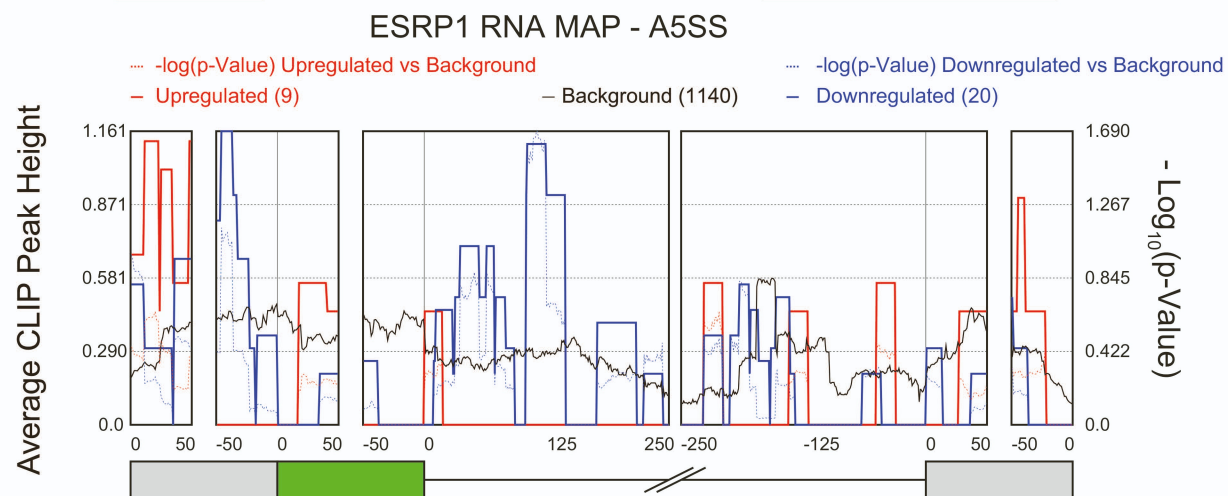
Supplementary Figure 5



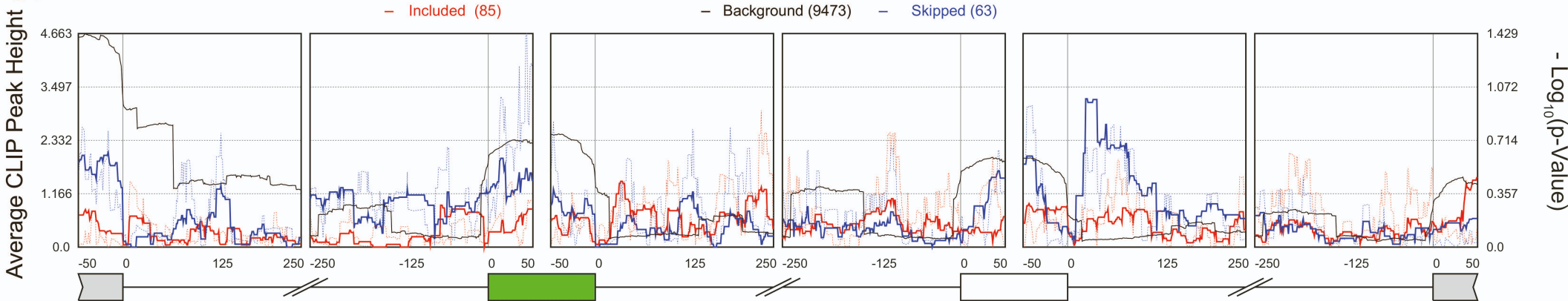
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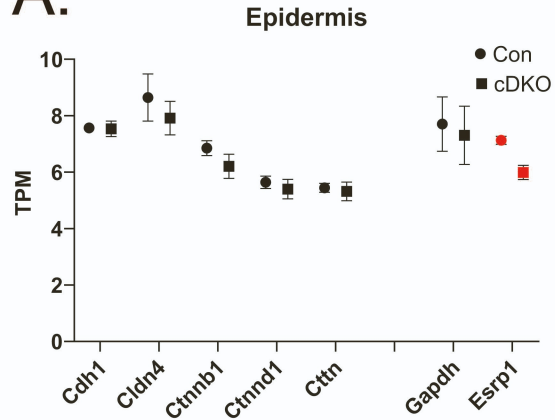
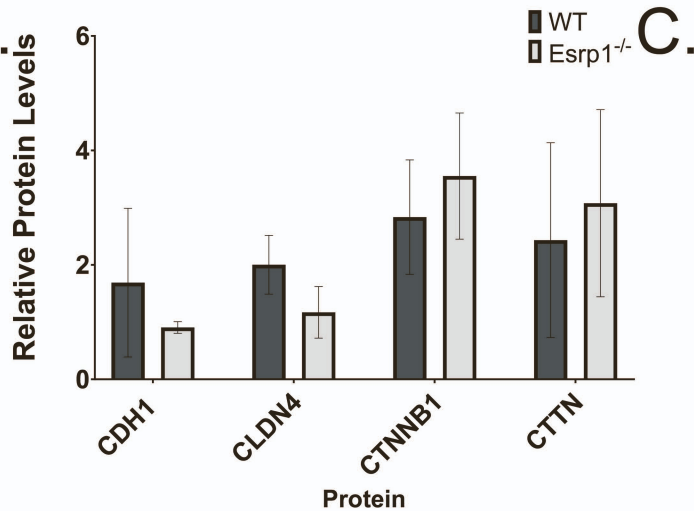


B.



C.



A.**B.****C.**