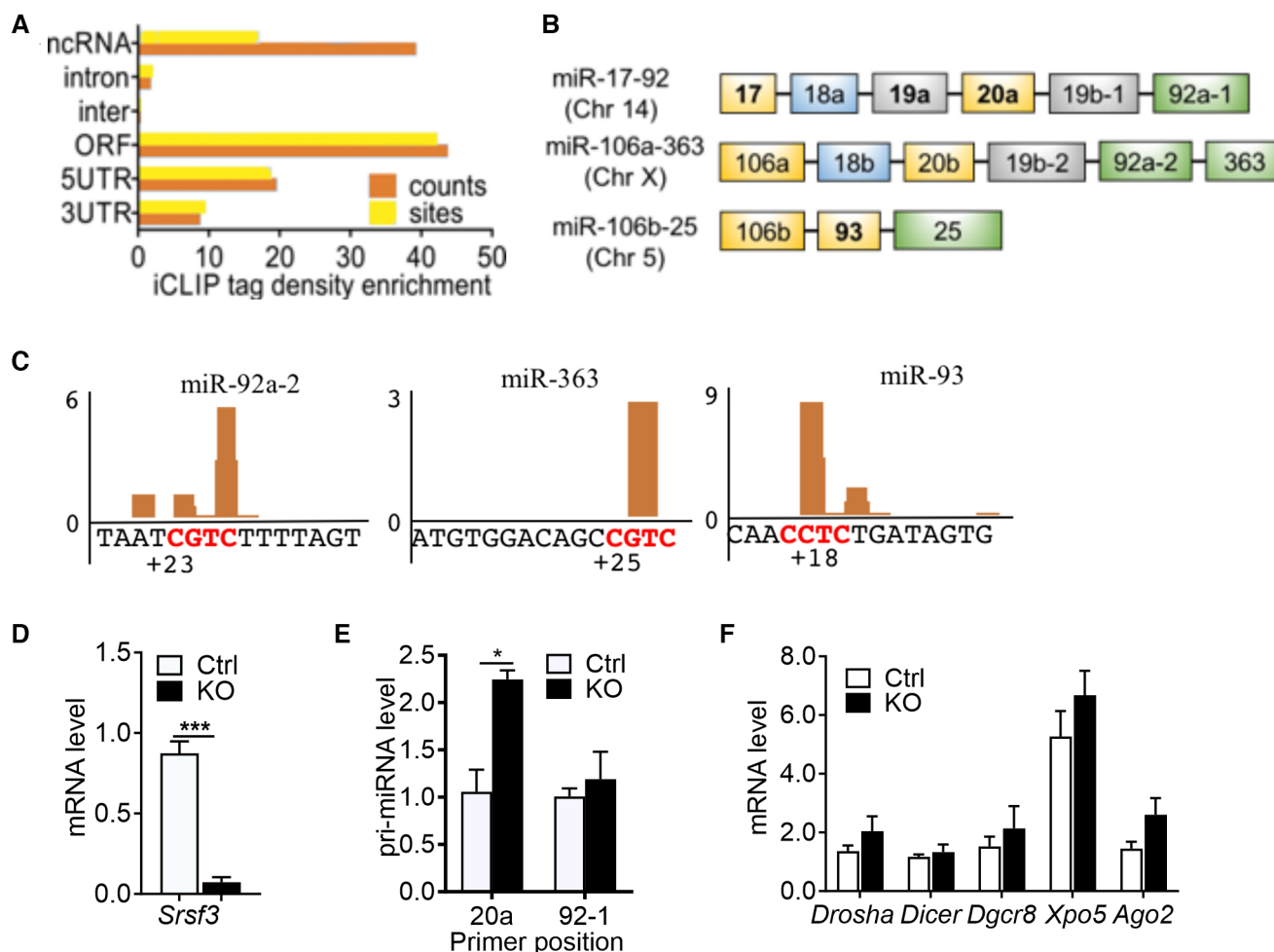


## Expanded View Figures



**Figure EV1. SRSF3 binds to miR-17-92 miRNA and its paralog clusters and affects the mature miRNA levels in pluripotent stem cells.**

A Distribution of significant SRSF3 crosslink sites (FDR < 0.05) over different transcript regions normalised to feature length. Data from (Ratnadiwakara et al, 2018).

B Schematic illustration of three paralog clusters miR-17-92, miR-106a-363 and miR-106b-25. The colours mark miRNAs of the same family. MicroRNAs bound by SRSF3 at 16–18 nt downstream of the Drosha cleavage site based on data in Ratnadiwakara et al (2018) are marked in bold.

C SRSF3 iCLIP binding peaks within the components of paralog clusters miR-106a-363 and miR-106b-25 in mouse ES cells. The CNNC motifs are marked in red colour. The 3p position is counted from the 3' Drosha cleavage site as described in (Auyeung et al, 2013), iCLIP data from (Ratnadiwakara et al, 2018).

D RT-qPCR quantification of *Srsf3* expression in *Srsf3*-knockout (KO) and control (Ctrl) iPSCs (\*\**P* < 0.001, two-tailed unpaired Student's *t*-test, data as mean ± SEM, *n* = 3, biological replicates).

E RT-qPCR quantification of pri-miRNA expression in *Srsf3*-KO (KO) and control (Ctrl) iPSCs. Two different primer pairs around miR-20a and miR-92-1 stem-loop regions were used (\**P* < 0.05, two-tailed unpaired Student's *t*-test, data as mean ± SEM, *n* = 3, biological replicates).

F RT-qPCR quantification of *Drosha*, *Dicer*, *Dgcr8*, *Xpo5* and *Ago2* mRNA levels in *Srsf3*-KO (KO) and control (Ctrl) iPSCs (*P* > 0.05 for all genes analysed, two-tailed unpaired Student's *t*-test, data as mean ± SEM, *n* = 3, biological replicates).

**Figure EV2. SRSF3 binding to the CNNC sites is essential for miR-17-92 processing.**

- A RT-qPCR analysis of wild-type (WT) miR17-92, 17/20a- $\Delta$ CNNC and total- $\Delta$ CNNC construct expression in HEK293T cells (data as mean  $\pm$  SEM,  $n = 4$ , biological replicates).
- B RT-qPCR analysis of wild-type (WT) miR17-92, 17/20a- $\Delta$ CNNC and total- $\Delta$ CNNC construct expression in HEK293T cells overexpressing SRSF3-GFP or GFP control (data as mean  $\pm$  SEM,  $n = 4$ , biological replicates).
- C–H TaqMan analysis of miR-17-92 miRNAs in HEK293 cells overexpressing SRSF3-GFP or GFP together with WT, 17/20a- $\Delta$ CNNC or total- $\Delta$ CNNC miR17-92 (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ , #all samples significantly different compared with control (Ctrl), One-Way ANOVA, data as mean  $\pm$  SEM,  $n = 4$ , biological replicates).
- I Co-Immunoprecipitation in SRSF3-GFP or GFP-only expressing LIM1215 cells. In = input, IP = immunoprecipitation. The immunoprecipitation was performed with an anti-GFP antibody and the Western blot detection with an anti-SRSF3 antibody.

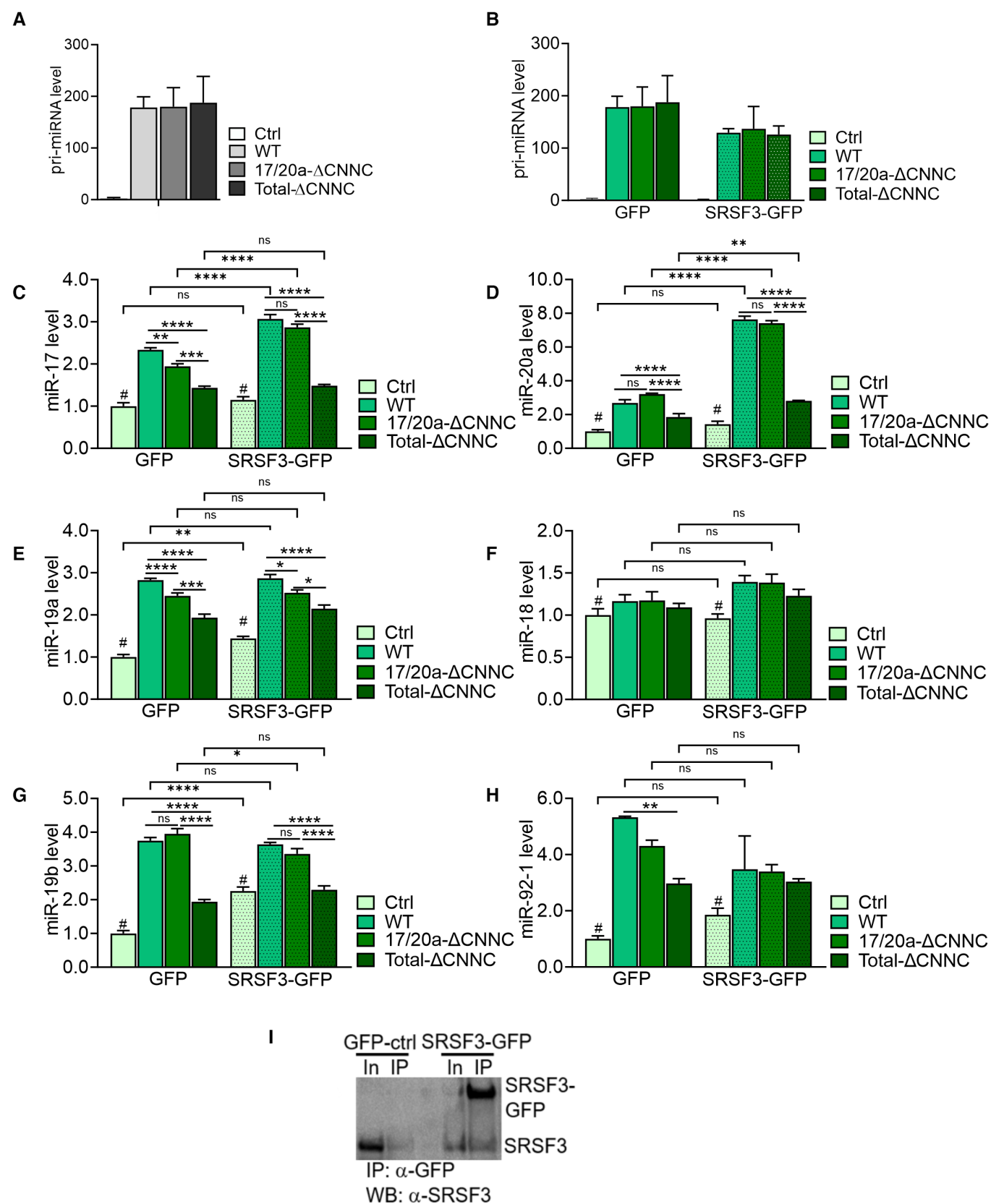


Figure EV2.

**Figure EV3. SRSF3 rearranges the miR-17-92 secondary structure through CNNC sites.**

- A WT miR-17-92 SHAPE reactivities in control (Ctrl) and SRSF3-KD cells based on ShapeMapper2. Red:  $\text{SHAPE} \geq 0.85$ , Yellow:  $0.85 > \text{SHAPE} > 0.4$  and Black:  $\text{SHAPE} \leq 0.4$ . The bar below represents miR-17-92 transcript with the miRbase predicted miRNA active strands marked in dark blue and star strands in light blue.
- B, C Comparison of 17/20a- $\Delta$ CNNC (B) and total- $\Delta$ CNNC (C) miR-17-92 SHAPE reactivities to WT miR-17-92 SHAPE reactivities. *First panel:* Mean reactivities, calculated over 11 nt sliding windows. *Second panel:*  $\Delta$ SHAPE reactivity differences between WT and 17/20a- $\Delta$ CNNC or total- $\Delta$ CNNC miR-17-92. Regions with higher reactivity in WT miR-17-92 are marked with blue shading and regions with higher reactivity in 17/20a- $\Delta$ CNNC or total- $\Delta$ CNNC with grey shading. *Third panel:* The 17/20a- $\Delta$ CNNC to WT miR-17-92 or total- $\Delta$ CNNC to WT miR-17-92 reactivity ratios. The boxes mark CNNC sites (black WT, red CNNC sites mutated).
- D Comparison of miR-17-92 SHAPE reactivities between 17/20a- $\Delta$ CNNC and total- $\Delta$ CNNC miR-17-92. *First panel:* Mean reactivities, calculated over 11 nt sliding windows. *Second panel:*  $\Delta$ SHAPE reactivity differences between 17/20a- $\Delta$ CNNC and total- $\Delta$ CNNC miR-17-92. Regions with higher reactivity in 17/20a- $\Delta$ CNNC are marked with light grey shading and regions with higher reactivity in total- $\Delta$ CNNC miR-17-92 are marked with dark grey shading. *Third panel:* The total- $\Delta$ CNNC to 17/20a- $\Delta$ CNNC reactivity ratios. The red boxes mark the mutated CNNC sites.
- E Visualisation and comparison of 17/20a- $\Delta$ CNNC miR-17-92 SHAPE reactivities in control and SRSF3-KD cells. *First panel:* Mean control (grey) and SRSF3-KD (brown) SHAPE reactivities, calculated over 11 nt sliding windows. *Second panel:*  $\Delta$ SHAPE reactivity differences between control and SRSF3-KD. Regions with higher reactivity in control are marked with grey shading and regions with higher reactivity in SRSF3-KD with brown shading. *Third panel:* The SRSF3-KD to control reactivity ratios. The boxes mark CNNC sites (black WT, red CNNC sites mutated).
- F Visualisation and comparison of WT miR-17-92 SHAPE reactivities in SRSF3-KD cells and total- $\Delta$ CNNC miR-17-92 SHAPE reactivities in control cells. *First panel:* Mean WT miR-17-92 SRSF3-KD (red) and total- $\Delta$ CNNC miR-17-92 control (grey) SHAPE reactivities, calculated over 11 nt sliding windows. *Second panel:*  $\Delta$ SHAPE reactivity differences between WT miR-17-92 SRSF3-KD and total- $\Delta$ CNNC miR-17-92 control. Regions with higher reactivity in WT miR-17-92 SRSF3-KD are marked with red shading and regions with higher reactivity in total- $\Delta$ CNNC miR-17-92 control with grey shading. *Third panel:* The total- $\Delta$ CNNC miR-17-92 control to WT miR-17-92 SRSF3-KD reactivity ratios. The red boxes mark the mutated CNNC sites.

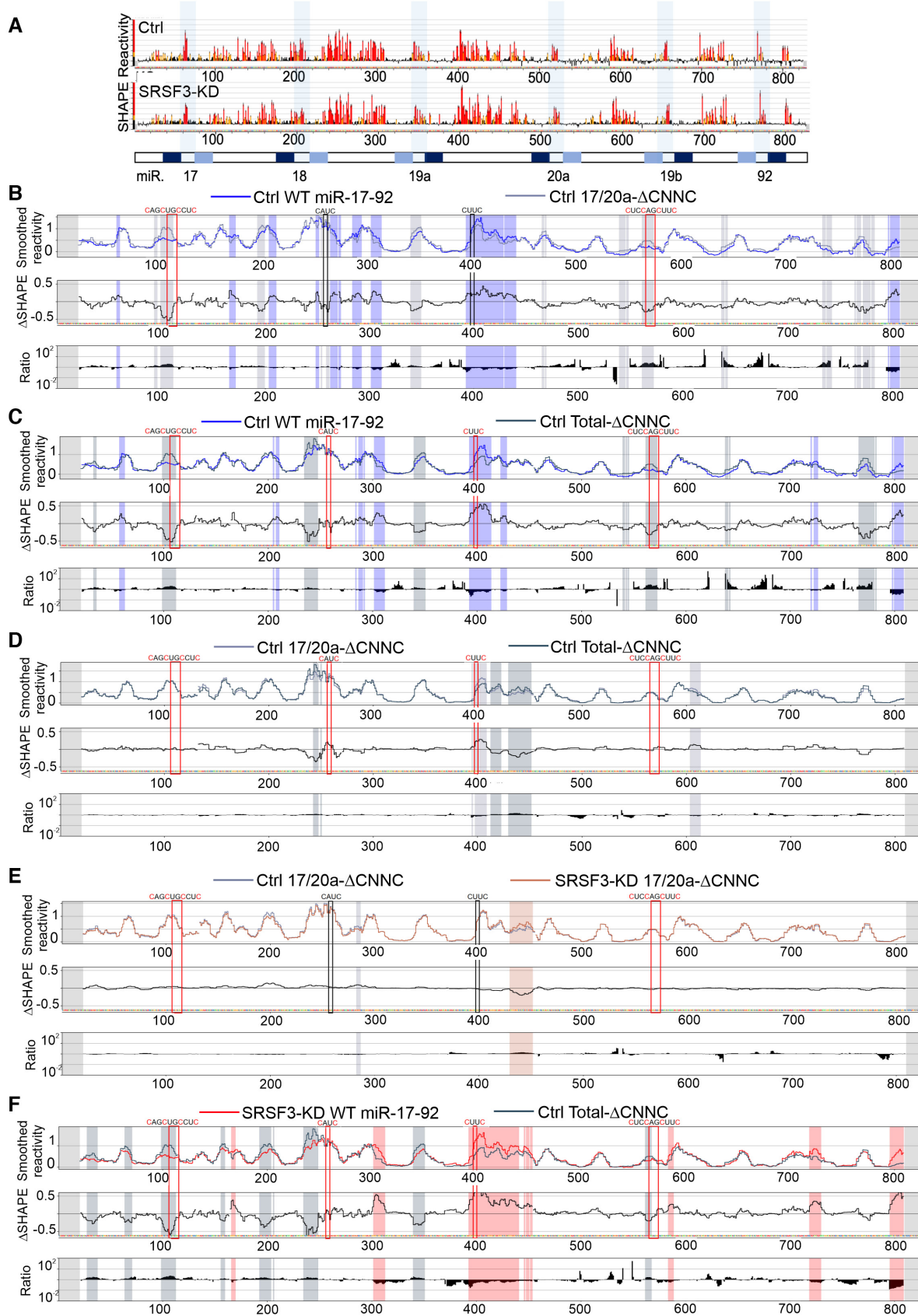
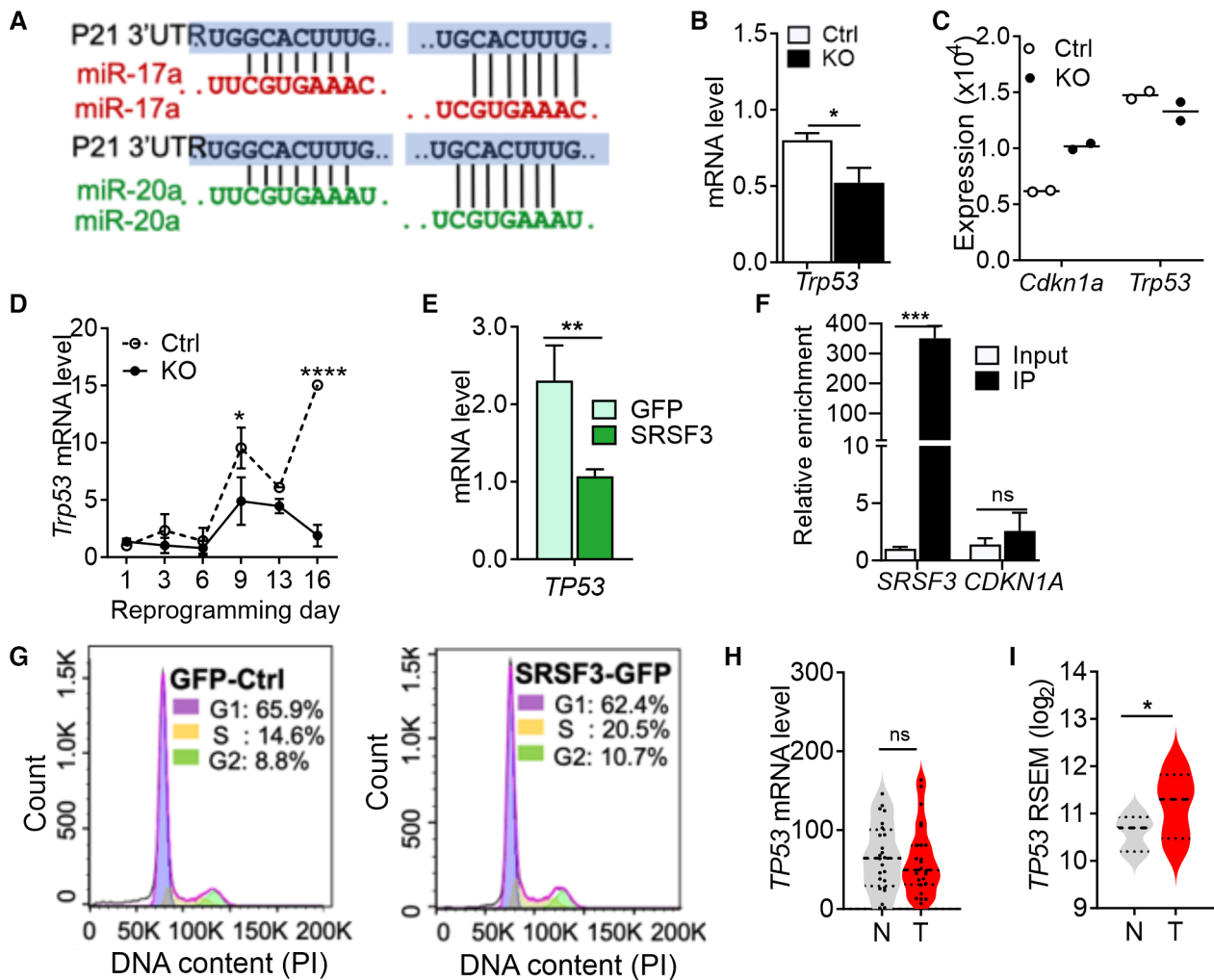


Figure EV3.



**Figure EV4. SRSF3 controls CDKN1A/p21 levels through miR-17/20a independent of p53.**

- A Schematic of miR-17 and miR-20a seed regions that target the *Cdkn1a* 3'UTR at two sites.
- B RT-qPCR quantification of *Trp53* (p53) mRNA levels in *Srsf3*-knockout (KO) and control (Ctrl) iPSCs (\* $P < 0.05$ , two-tailed unpaired Student's *t*-test, data as mean  $\pm$  SEM,  $n = 3$ , biological replicates).
- C Quantification of *Cdkn1a* and *Trp53* expression from *Srsf3*-KO and Ctrl RNA-sequencing data from (Ratnadiwakara et al, 2018; data as mean,  $n = 2$ , biological replicates).
- D Quantification of *Trp53* mRNA expression by RT-qPCR during reprogramming in *Srsf3*-KO (KO) and control (Ctrl) cells (\* $P < 0.05$ , \*\*\*\* $P < 0.0001$ , two-way ANOVA, data as mean  $\pm$  SEM,  $n = 3$ , biological replicates).
- E RT-qPCR quantification of *TP53* (p53) mRNA levels in SRSF3 overexpressing (SRSF3-GFP) and control (GFP) LIM1215 cells (\*\* $P < 0.01$ , two-tailed unpaired Student's *t*-test, data as mean  $\pm$  SEM,  $n = 3$ , biological replicates).
- F RNA immunoprecipitation (IP) of SRSF3 (positive control) and *CDKN1A* mRNAs in GFP-only and SRSF3-GFP expressing LIM1215 cells (\*\*\* $P < 0.001$ , Two-tailed unpaired Student's *t*-test, data as mean  $\pm$  SEM,  $n = 3$ , biological replicates).
- G Representative histograms of propidium iodide-stained control (GFP) and SRSF3-T2A-GFP overexpressing (SRSF3-GFP) LIM1215 cells analysed by flow cytometry. Histograms were generated using cell cycle analysis tool in FlowJo software (Tree Star). Per cent cells in each phase are indicated in the inset.
- H RT-qPCR quantification of *TP53* mRNA levels in colorectal tumours and their paired normal samples (ns = not significant, two-tailed unpaired Student's *t*-test,  $n = 25$ , biological replicates, central band = median, dotted lines = 25<sup>th</sup> and 75<sup>th</sup> percentile).
- I Relative *TP53* expression in TCGA COAD data (version 2016\_01\_28, tumour  $n = 459$  and normal  $n = 41$ , \* $P < 0.05$ , two-tailed unpaired Student's *t*-test, biological replicates, central band = median, dotted lines = 25<sup>th</sup> and 75<sup>th</sup> percentile).