Supplemental Material

# RNA-binding proteins regulate post-transcriptional responses to TGF-β to coordinate function and mesenchymal activation of murine endothelial cells

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Supplemental Figures

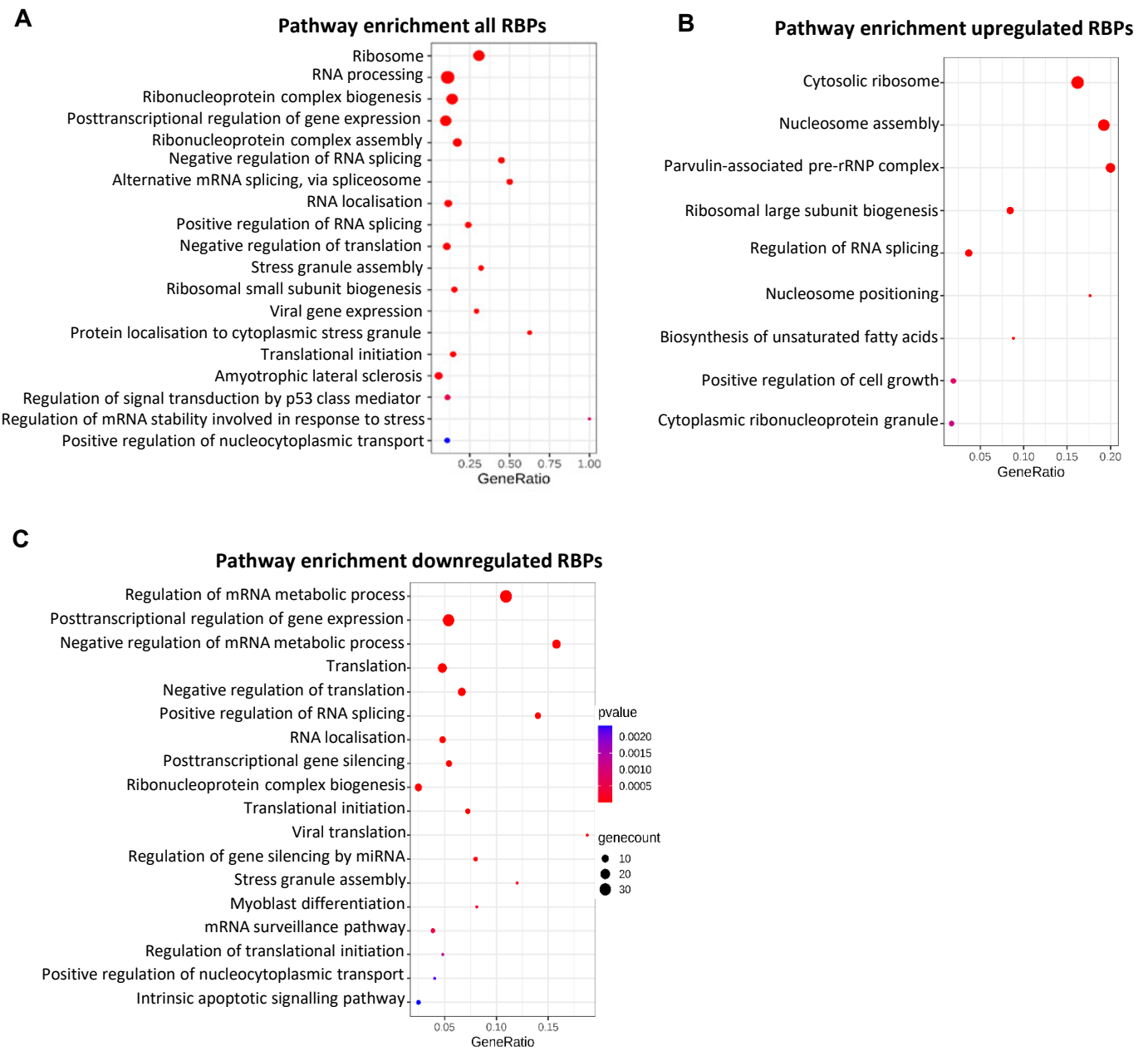



**Figure S1. Ontological analysis of endothelial RBPs (related to figure 1).** A. Pathway (KEGG and GO biological process) enrichment of all RBPs identified by RNA interactome capture of MCECs. B. Pathway (KEGG and GO biological process) enrichment of RBPs with increased RNA binding upon TGF-β stimulation (10 ng/ml, 24 h) (fold change 100% or greater) as identified by RNA interactome capture. C. Pathway (KEGG and GO biological process) enrichment of RBPs with decreased RNA binding upon TGF-β stimulation (10 ng/ml, 24 h) (fold change 100% or greater) as identified by RNA interactome capture.

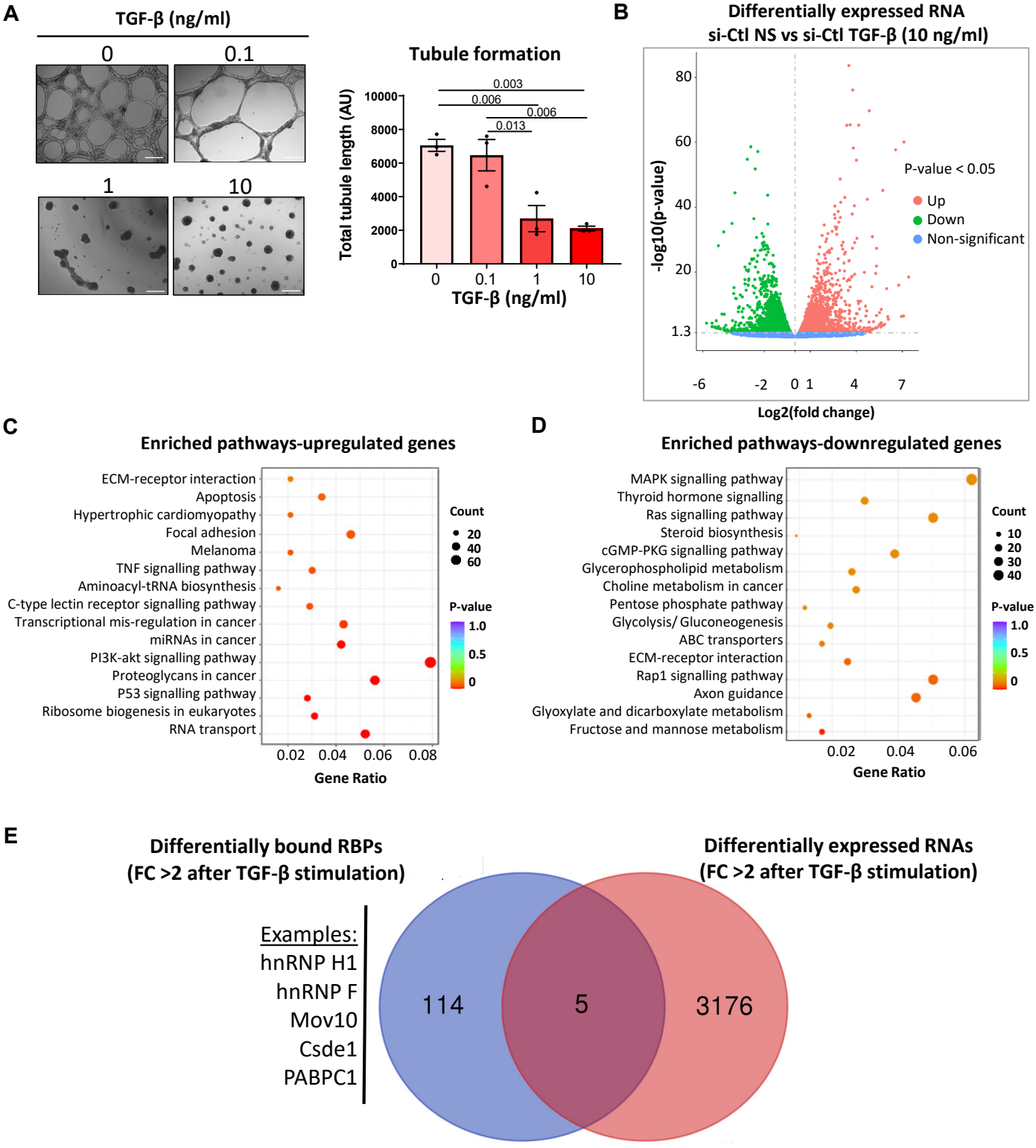



**Figure S2. Effects of TGF-β on MCECs and mesenchymal activation A. Effects of TGF-β on tubule formation.** MCECs were incubated with TGF-β for 24 hours followed by tubule formation assay. Quantifications reflect total tubule length in visible field, n=3, data shown as average ±SEM, significance assessed with one-way ANOVA and Tukey's multiple comparison test, significance shown to three significant figures. Scale bar 250 µm. **C. Pathway enrichment differentially expressed genes.** Dot plot showing the enriched pathways in differentially expressed genes following TGF-β stimulation**. D. Pathway enrichment upregulated genes.** Dot plot showing selected enriched pathways of upregulated genes following TGF-β stimulation. **F. Pathway enrichment downregulated genes.** Dot plot showing selected enriched pathways of downregulated genes following TGF-β stimulation. **E. Overlap between TGF-β regulated RBPs and differential expressed genes upon TGF-β stimulation.** Overlap of RBPs which showed a greater than two fold change in RNA binding upon TGF-β stimulation (10 ng/ml, 24 h) with differentially expressed RNAs (RNA seq.) following TGF-β stimulation (10 ng/ml, 24 h).

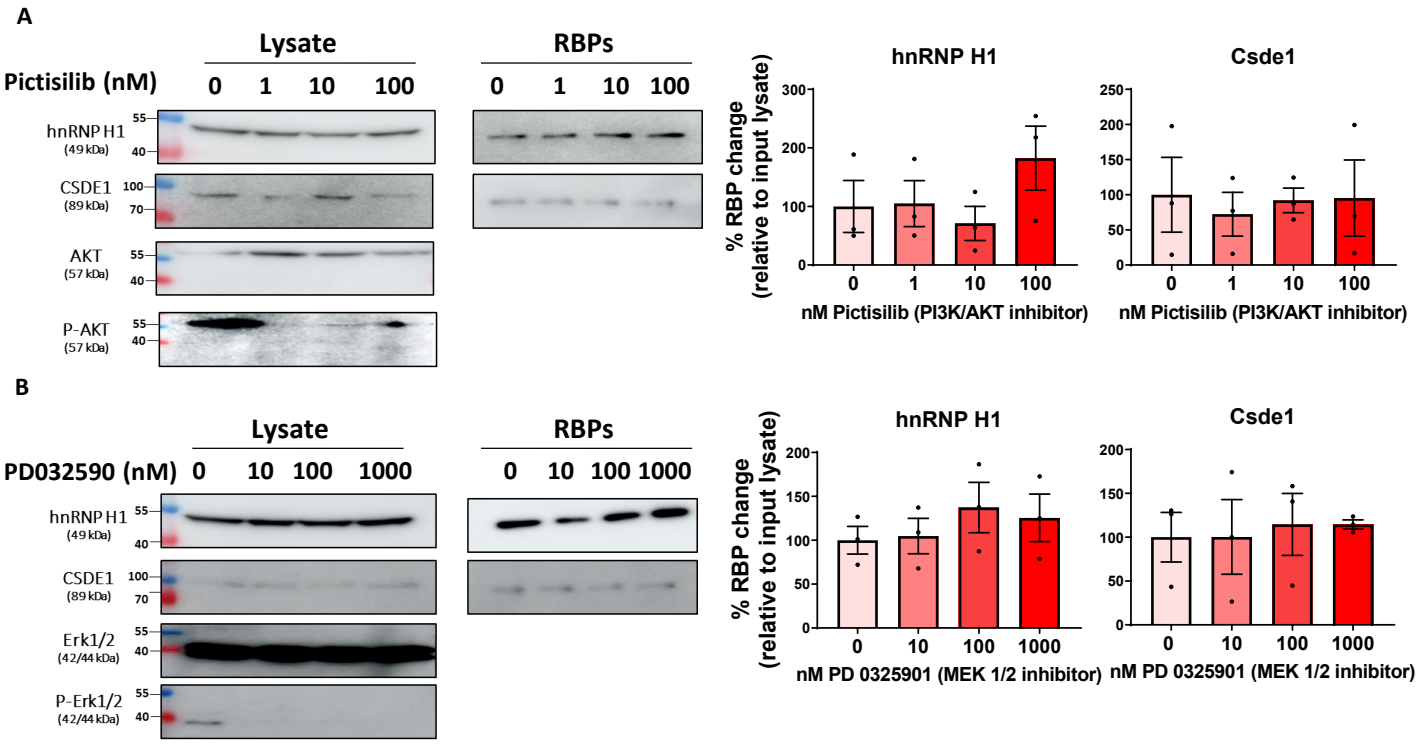



**Figure S3. Mechanism of RNA binding activity regulation. A. Effects of PI3K/AKT inhibition on RNA binding**. MCECs were incubated in 10 ng/ml TGF-β for 24 hours in the presence of increasing concentrations of Pictilisib, a selective inhibitor of the PI3K/AKT signalling pathway, followed by UV-crosslinking and RNA interactome capture. Quantifications reflect changes in abundance of proteins in RNA interactome isolates, normalised to input lysate, n=3, error bars show average ±SEM, no statistical significance after one-way ANOVA and Tukey's multiple comparison test. **B. Effects of ERK inhibition on RNA binding.** MCECs were incubated in 10 ng/ml TGF-β for 24 hours in the presence of increasing concentrations of PD 0325901, a selective inhibitor of the MEK1 and MEK2, followed by UV-crosslinking and RNA interactome capture. Quantifications reflect changes in abundance of proteins in RNA interactome isolates, normalised to input lysate, n=3, data shown as average ±SEM, no statistical significance after one-way ANOVA and Tukey's multiple comparison test.

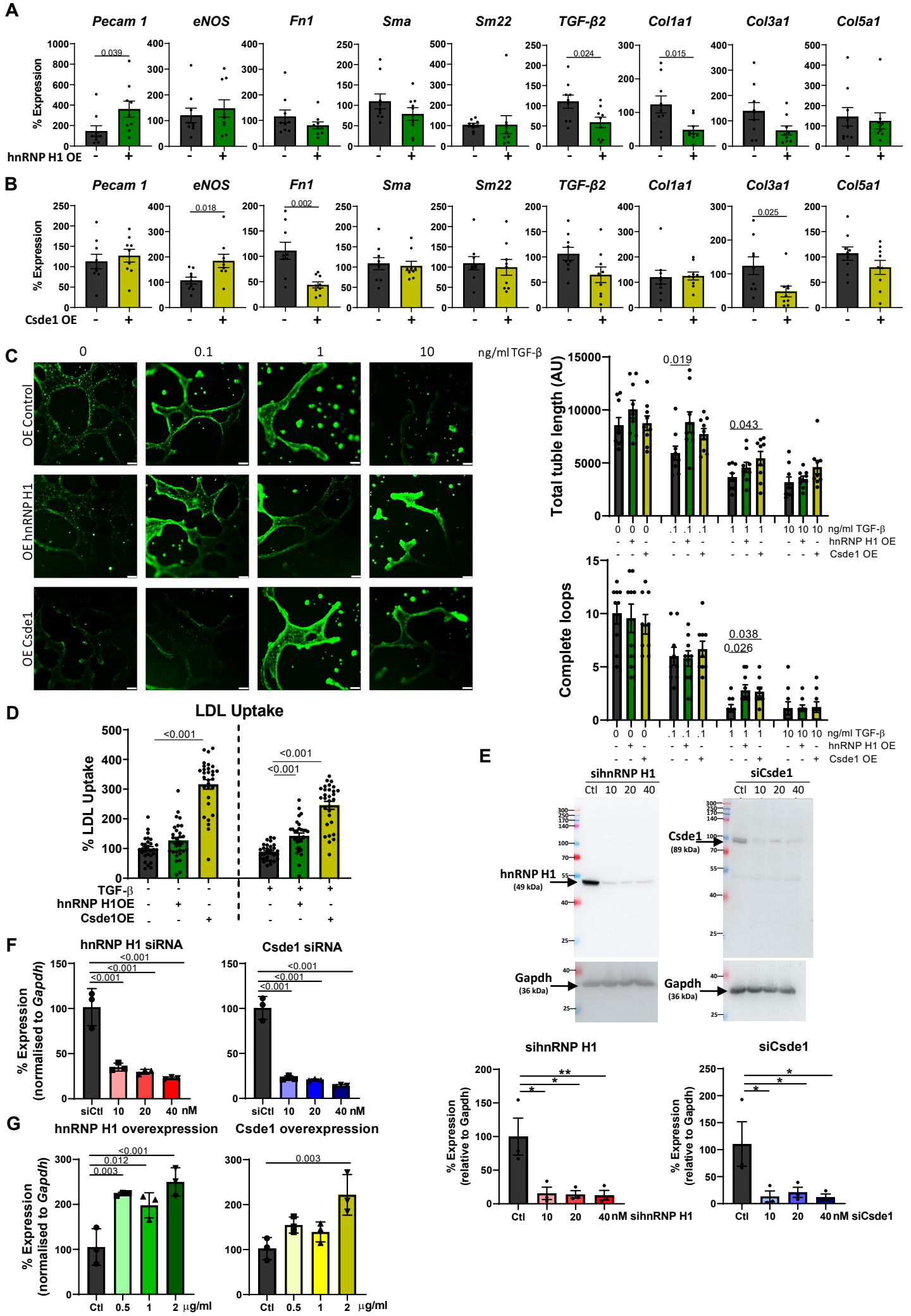

A
Pecam 1
eNOS
Fn1
Sma
Sm22
TGF-β2
Col1a1
Col3a1
Col5a1
% Expression
0.039
0.024
0.015
hnRNP H1 OE
B
0.018
0.002
0.025
Csde1 OE
C
0
0.1
1
10
ng/ml TGF-β
OE Control
OE hnRNP H1
OE Csde1
Total tuble length (AU)
0.019
0.043
Complete loops
0.038
0.026
hnRNP H1 OE
Csde1 OE
D
LDL Uptake
% LDL Uptake
<0.001
TGF-β
hnRNP H1OE
Csde1OE
E
sihnRNP H1
siCsde1
Ctl 10 20 40
hnRNP H1
(49 kDa)
Csde1
(89 kDa)
Gapdh
(36 kDa)
% Expression
(relative to Gapdh)
Ctl 10 20 40 nM sihnRNP H1
Ctl 10 20 40 nM siCsde1
F
hnRNP H1 siRNA
Csde1 siRNA
% Expression
(normalised to Gapdh)
siCtl 10 20 40 nM
G
hnRNP H1 overexpression
Csde1 overexpression
0.012
0.003
Ctl 0.5 1 2 µg/ml

**Figure S4. A. Effects of hnRNP H1 overexpression on mesenchymal marker gene expression.** MCECs were transfected with hnRNP H1 OE construct (48 h) and TGF-β stimulation (10 ng/ml, 24 h), followed by RNA isolation and RT-qPCR analysis of the expression of selected mesenchymal genes. Expression normalised to *Gapdh*, n=3, each in triplicate, data shown as average ±SEM, normality assessed by Shapiro Wilk and significance assessed by unpaired Student's t-test, significance shown to three significant figures. **B. Effects of Csde1 overexpression on mesenchymal marker gene expression.** MCECs were transfected with Csde1 OE construct (48 h) and TGF-β stimulation (10 ng/ml, 24 h), followed by RNA isolation and RT-qPCR analysis of the expression of selected mesenchymal genes. All expression normalised to *Gapdh*, n=3, each in triplicate, data shown as average ±SEM, normality assessed by Shapiro Wilk and significance assessed by unpaired Student's t-test, significance shown to three significant figures. **C. Effects of hnRNP H1 and Csde1 overexpression on tubule formation.** hnRNP H1 and Csde1 were overexpressed (48 h) in MCECs incubated with TGF-β (24). Cells were cultured on matrigel membrane (24 h), flourescently labelled and tubule formation assessed by microscopy. Quantifications represent average total tubule length and number of complete loops. Representative images (n=3 triplicates scale bar 100μm). Data shown as average ± SEM, normality assessed by Shapiro Wilk, significance assessed by one way-ANOVA with Dunnett's multiple comparison, significance shown to three significant figures. **D. Effects of hnRNP H1 and Csde1 overexpression on LDL uptake.** hnRNP H1 and Csde1 were overexpressed (48 h) +/- TGF-β stimulation (10 ng/ml, 24 h). MCECs were then incubated in fluorescently labelled LDL and uptake assessed and quantified by fluorescence microscopy. Data shown as average ±SEM. Normality assessed by Shapiro Wilk, significance assessed by one way-ANOVA with Dunnett's multiple comparison, significance shown to three significant figures. **E. Optimisation/validation of siRNA knockdown of hnRNP H1 and Csde1 (Western blot).** MCECs were transfected with increasing concentrations of sihnRNP H1 or siCsde1 siRNA and expression assessed after 48 hours by Western blot. Expression relative to *Gapdh,* n=3, data shown as average ±SEM, normality assessed by Shapiro Wilk, significance assessed by one way-ANOVA with Dunnett's multiple comparison, significance shown to three significant figures. **F. Optimisation/validation of siRNA knockdown of hnRNP H1 and Csde1 (RT-qPCR).** MCECs were transfected with increasing concentrations of sihnRNP H1 or siCsde1 siRNA and expression assessed after 48 hours by RT-qPCR. Expression relative to *Gapdh,* n=3, data shown as average ±SEM, normality assessed by Shapiro Wilk, significance assessed by one way-ANOVA with Dunnett's multiple comparison, significance shown to three significant figures. **G. Optimisation/validation of hnRNP H1 and Csde1 overexpression.** MCECs were transfected with increasing concentrations of hnRNP H1 or Csde1 constructs and expression assessed after 48 hours by RT-qPCR. Expression relative to *Gapdh,* n=3, data shown as average ±SEM, normality assessed by Shapiro Wilk, significance assessed by one way-ANOVA with Dunnett's multiple comparison, significance shown to three significant figures.

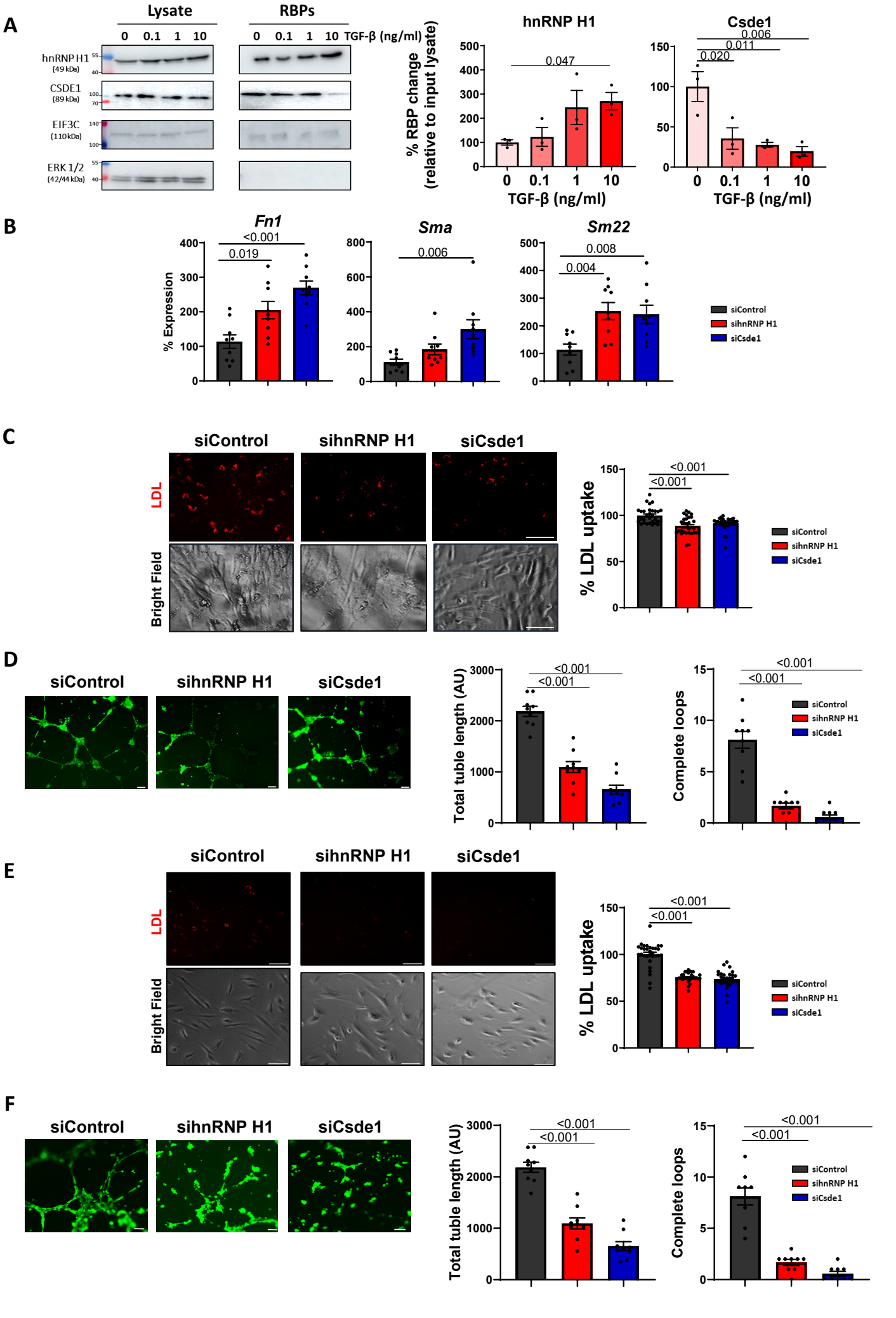

A
Lysate
RBPs
0 0.1 1 10
0 0.1 1 10 TGF-β (ng/ml)
hnRNP H1 (49 kDa)
CSDE1 (89 kDa)
EIF3C (110 kDa)
ERK 1/2 (42/44 kDa)
% RBP change (relative to input lysate)
hnRNP H1
0.047
Csde1
0.006
0.011
0.020
TGF-β (ng/ml)
B
Fn1
<0.001
0.019
Sma
0.006
Sm22
0.008
0.004
% Expression
siControl
sihnRNP H1
siCsde1
C
siControl
sihnRNP H1
siCsde1
LDL
Bright Field
<0.001
<0.001
% LDL uptake
D
siControl
sihnRNP H1
siCsde1
Total tuble length (AU)
<0.001
<0.001
Complete loops
<0.001
<0.001
E
siControl
sihnRNP H1
siCsde1
LDL
Bright Field
<0.001
<0.001
% LDL uptake
F
siControl
sihnRNP H1
siCsde1
Total tuble length (AU)
<0.001
<0.001
Complete loops
<0.001
<0.001

**Figure S5. hnRNP H1 and Csde1 in primary human endothelial cells. A. TGF-β regulated changes in RNA binding are conserved in HUVECs.** Human Umbilical Vein Endothelial Cells (HUVECs) were incubated in increasing TGF-β (24 h) followed by UV-cross-linking and RIC. Quantifications reflect changes in abundance in RIC isolates, normalised to input lysate, n=3, data shown as average ±SEM. Normality assessed by Shapiro Wilk, significance assessed by one-way ANOVA with a Dunnett's multiple comparison test, significance shown to three significant figures. **B. Effects of hnRNP H1 and Csde1 knockdown on mesenchymal gene expression in HUVECs.** hnRNP H1 or Csde1 were knocked down in HUVECs by siRNA for 48 hours followed by RT-qPCR analysis of selected mesenchymal marker genes. Expression normalised to *Gapdh,* n=3 each in triplicate, data shown as average ±SEM, normality assessed by Shapiro Wilk, significance assessed by one-way ANOVA with a Dunnett's multiple comparison test, significance shown to three significant figures. **C. Effects of hnRNP H1 and Csde1 knockdown on LDL uptake in HUVECs.** hnRNP H1 and Csde1 were knocked down in HUVECs (siRNA, 48 h), cells were incubated in fluorescently labelled LDL and uptake assessed by fluorescence microscopy. Representative images (n=3 experiments, scale bar 100μm)**.** Data shown as average ± SEM. n=3 (10 quantifications per replicate), normality assessed by Shapiro Wilk, significance assessed by one-way ANOVA with a Dunnett's multiple comparison test, significance shown to three significant figures. **D. Effects of hnRNP H1 and Csde1 knockdown on tubule formation in HUVECs.** hnRNP H1 and Csde1 were knocked down in HUVECs (siRNA, 48 h), cells were plated on matrigel (24 h), fluorescently labelled and tubule formation assessed by microscopy. Quantifications represent average total tubule length and number of complete loops. Representative images (n=3 triplicates scale bar 100μm). Data shown as average ± SEM, normality assessed by Shapiro Wilk, significance assessed by one-way ANOVA with a Dunnett's multiple comparison test, significance shown to three significant figures. **E. Effects of hnRNP H1 and Csde1 knockdown on LDL uptake in HCMECs .** hnRNP H1 and Csde1 were knocked down in HCMECs (siRNA, 48 h), cells were incubated in fluorescently labelled LDL and uptake assessed by fluorescence microscopy. Representative images (n=3 experiments, scale bar 100μm)**.** Data shown as average ± SEM. n=3 (10 quantifications per replicate), significance assessed by a Krucksal-Wallis test, significance shown to three significant figures.

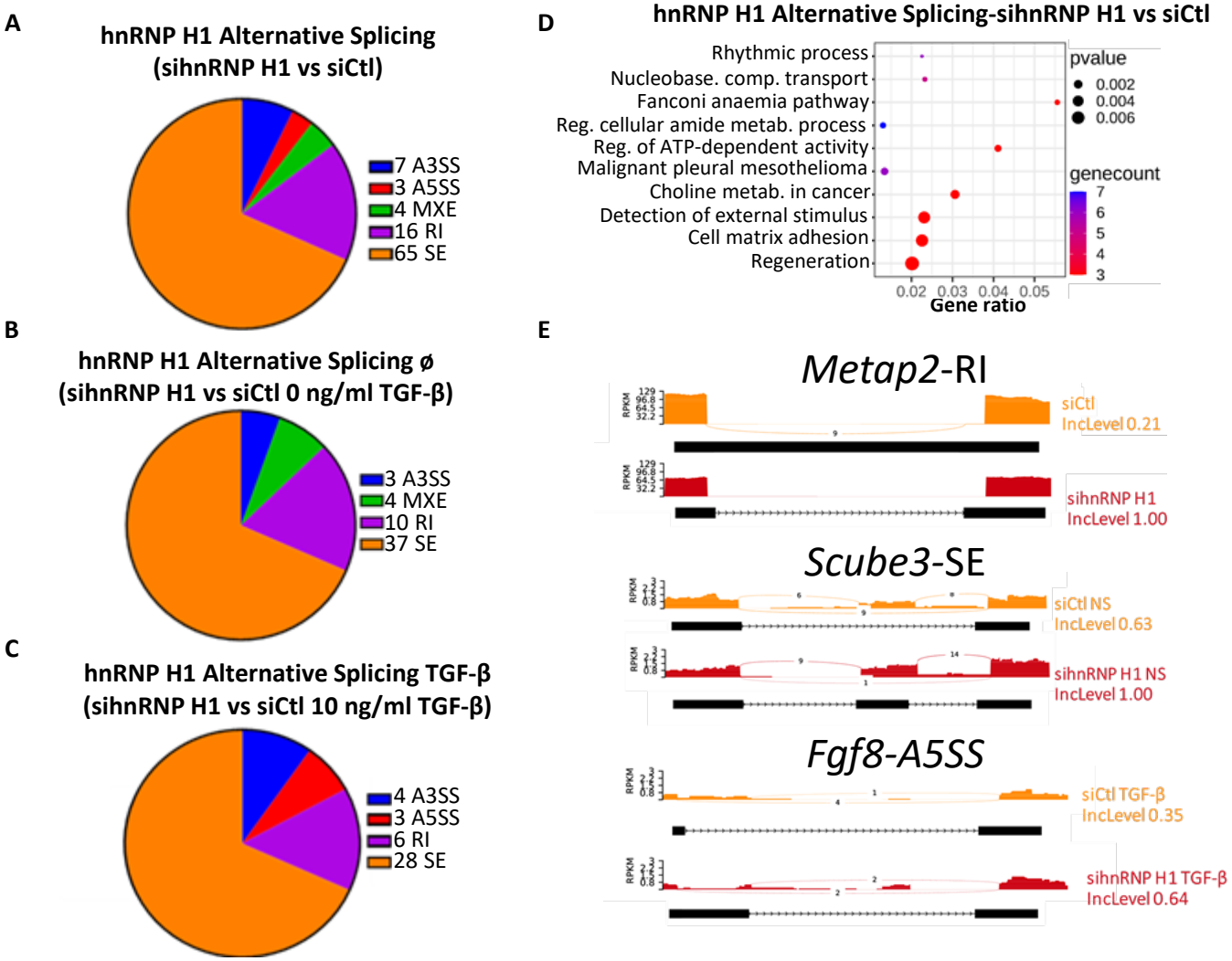



**Figure S6. Effects of hnRNP H1 knockdown on alternative splicing patterns. A. Significant alternative splicing events following hnRNP H1 knockdown.** Pie chart showing the significant alternative splicing changes upon sihnRNP H1 knockdown in both the presence and absence of TGF-β stimulation as detected by RNA sequencing between siCtl vs sihnRNP H1 conditions (FDR < 0.05). **B. Significant alternative splicing events following hnRNP H1 knockdown in the absence of TGF-β stimulation.** Pie chart showing the significant alternative splicing events upon knockdown of hnRNP H1 under basal conditions (siCtl 0 ng/ml TGF-β vs sihnRNP H1 0 ng/ml TGF-β) as detected by RNA sequencing (FDR < 0.05). **C. Significant alternative splicing events following hnRNP H1 knockdown in the presence of TGF-β stimulation.** Pie chart showing the significant alternative splicing events upon knockdown of hnRNP H1 in the presence of TGF-β stimulation (siCtl 10 ng/ml TGF-β vs sihnRNP H1 10 ng/ml TGF-β) as detected by RNA sequencing (FDR < 0.05). **D. Alternative splicing following sihnRNP H1 knockdown in the presence and absence of TGF-β stimulation.** Dot plot showing enriched pathways in genes which showed significant changes in splicing (FDR <0.05) in sihnRNP H1 vs siCtl samples in both the presence and absence of TGF-β stimulation. **E. Representative examples of alternative splicing events.** Representative splicing maps of differentially spliced transcripts upon knockdown of hnRNP H1.

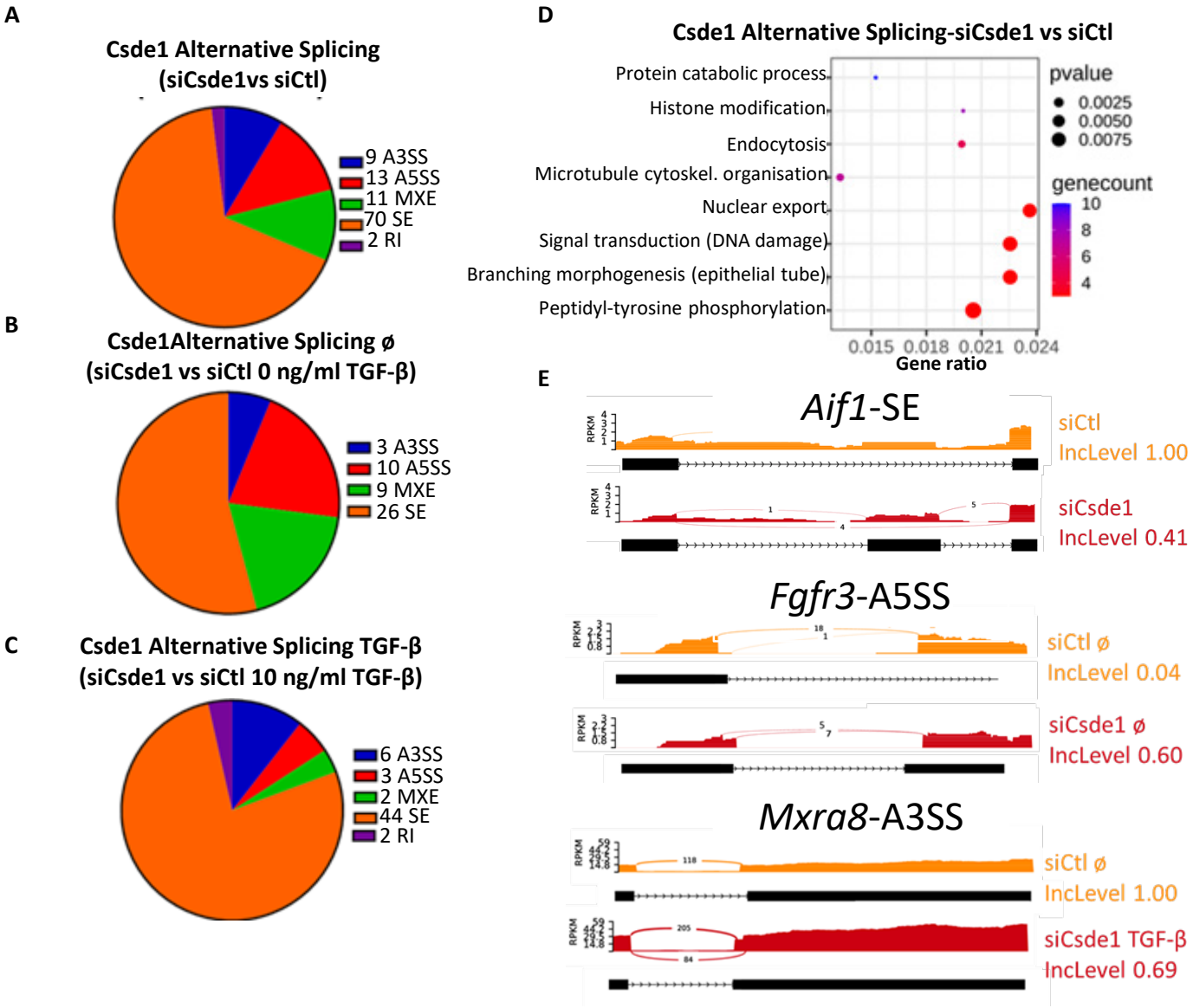



**Figure S7. Effects of Csde1 knockdown on alternative splicing patterns. A. Significant alternative splicing events following Csde1 knockdown.** Pie chart showing the significant alternative splicing changes upon siCsde1 knockdown in both the presence and absence of TGF-β stimulation as detected by RNA sequencing between siCtl vs siCSde1 conditions (FDR < 0.05). **B. Significant alternative splicing events following Csde1 knockdown in the absence of TGF-β stimulation.** Pie chart showing the significant alternative splicing events upon knockdown of Csde1 under basal conditions (siCtl 0 ng/ml TGF-β vs siCsde1 H1 0 ng/ml TGF-β) as detected by RNA sequencing (FDR < 0.05). **C. Significant alternative splicing events following Csde1 knockdown in the presence of TGF-β stimulation.** Pie chart showing the significant alternative splicing events upon knockdown of Csde1 in the presence of TGF-β stimulation (siCtl 10 ng/ml TGF-β vs siCsde1 10 ng/ml TGF-β) as detected by RNA sequencing (FDR < 0.05). **D. Alternative splicing following siCsde1 knockdown in the presence and absence of TGF-β stimulation.** Dot plot showing enriched pathways in genes which showed significant changes in splicing (FDR <0.05) in siCsde1 vs siCtl samples in both the presence and absence of TGF-β stimulation. **E. Representative examples of alternative splicing events.** Representative splicing maps of differentially spliced transcripts upon knockdown of Csde1.

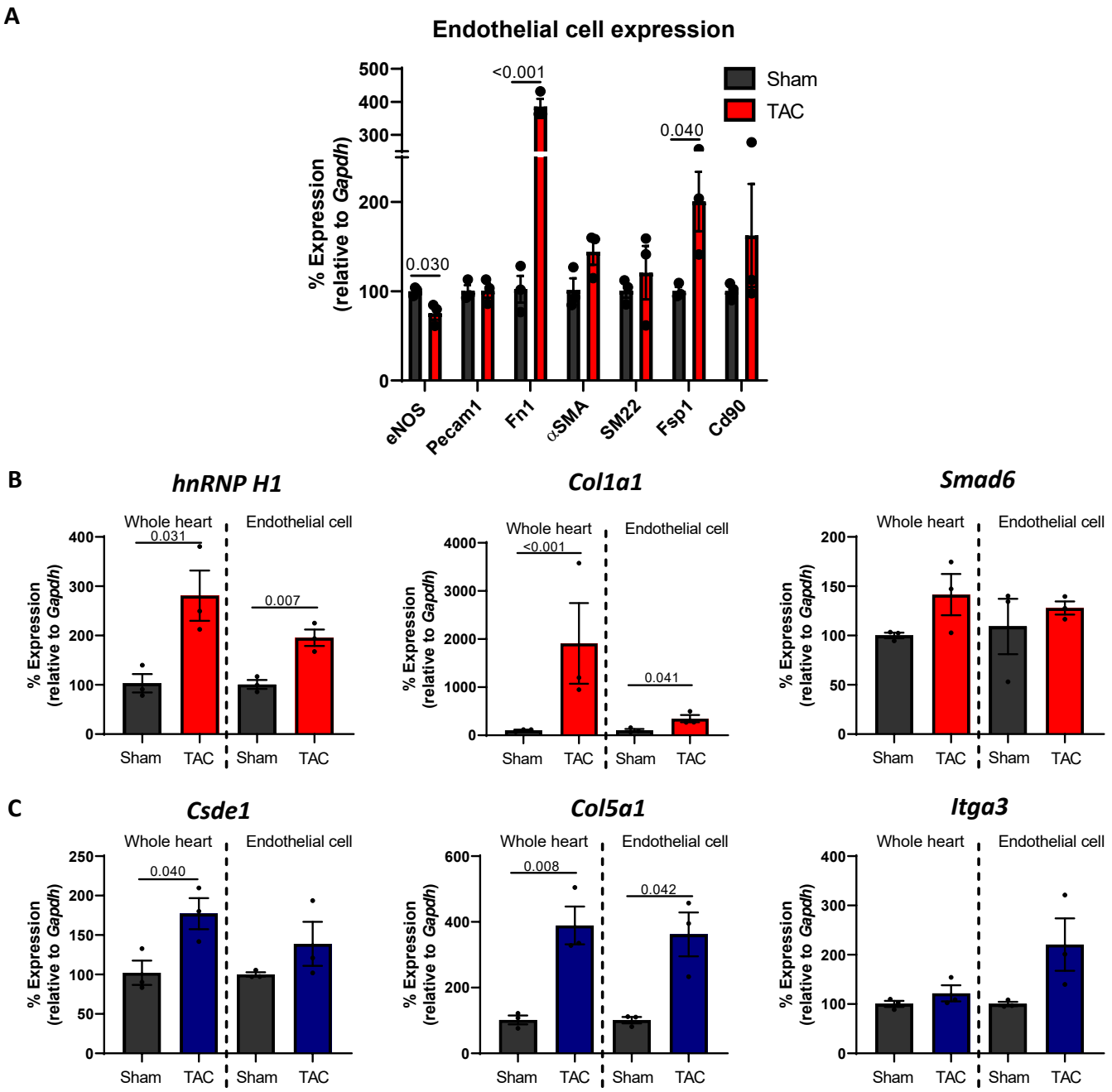



**Figure S8. A. Expression of selected EndoMT marker genes in endothelial cells after TAC.** RT-qPCR analysis of genes in isolated cardiac endothelial cells from sham and two week TAC operated mice. Data shown as average ± SEM, normality assessed by Shapiro Wilk test, significance assessed by Student's t-test, significance shown to three significant figures. **B. Expression of hnRNP H1 and target genes in whole heart and endothelial cells after TAC.** RT-qPCR analysis of *hnRNP H1, Col1a1* and *Smad6* expression in whole heart and isolated endothelial cells from sham and two week TAC operated mice. Data shown as average ± SEM, normality assessed by Shapiro Wilk test, significance assessed by Student's t-test, significance shown to three significant figures. **C. Expression of *Csde1* and target genes in whole heart and endothelial cells after TAC.** RT-qPCR analysis of *Csde1*, *Col5a1* and *Itga3* expression in whole heart and isolated endothelial cells from sham and two week TAC operated mice. Data shown as average ± SEM, normality assessed by Shapiro Wilk test, significance assessed by Student's t-test, significance shown to three significant figures.

**Supplementary Excel tables**

**Supplementary Table 1.**

Proteomic analysis of the TGF-β regulated RNA interactome (related to Figure 1).

**Supplementary Table 2.**

RIP analysis of TGF-β regulated RNA binding patterns of hnRNP H1 (related to Figure 4).

**Supplementary Table 3.**

RNA sequencing analysis of differential RNA expression following si-hnRNP H1 knockdown (related to Figure 4).

**Supplementary Table 4.**

RIP analysis of TGF-β regulated RNA binding patterns of Csde1 (related to Figure 6).

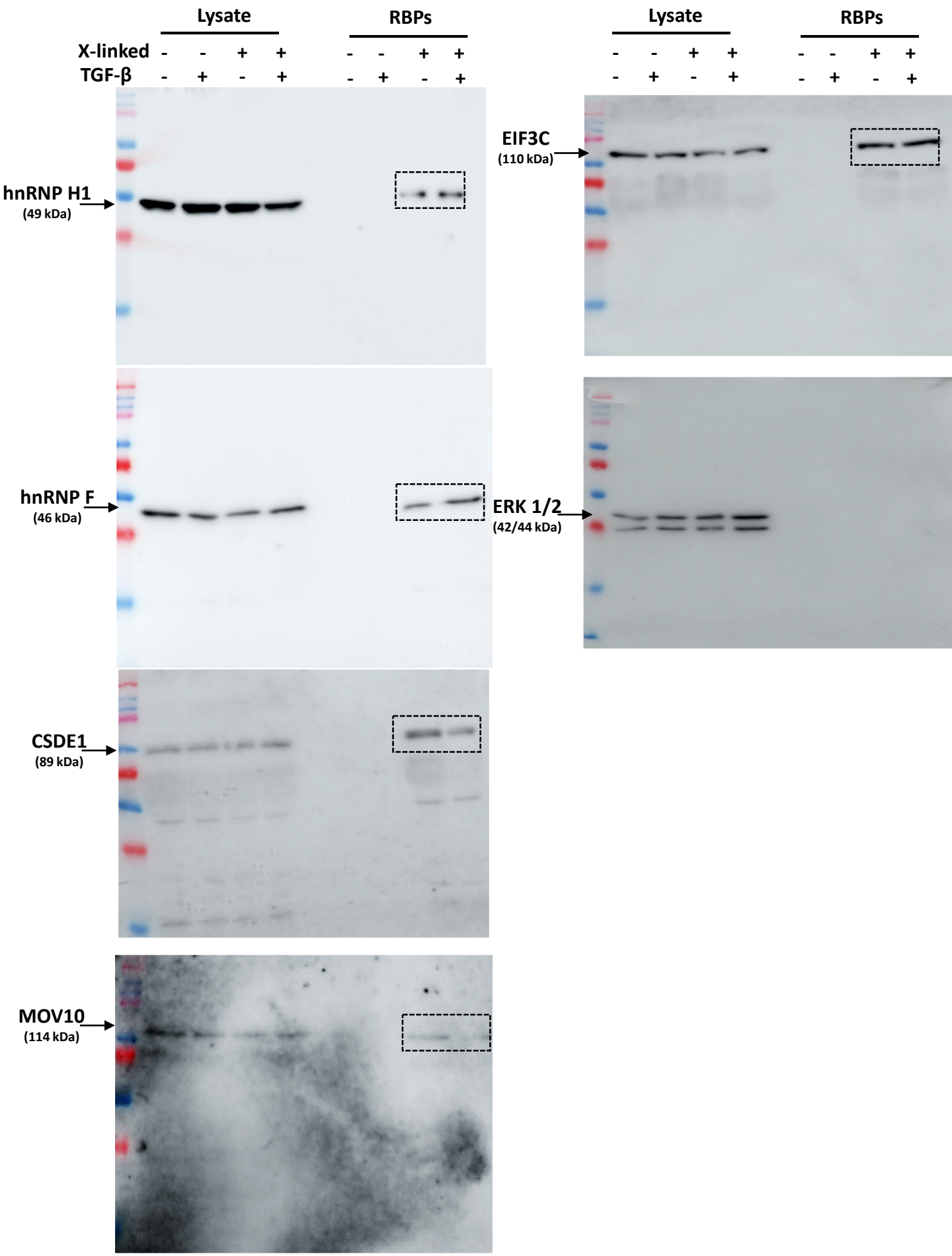
**Supplementary Table 5.**

RNA sequencing analysis of differential RNA expression following si-Csde1 knockdown (related to Figure 6).

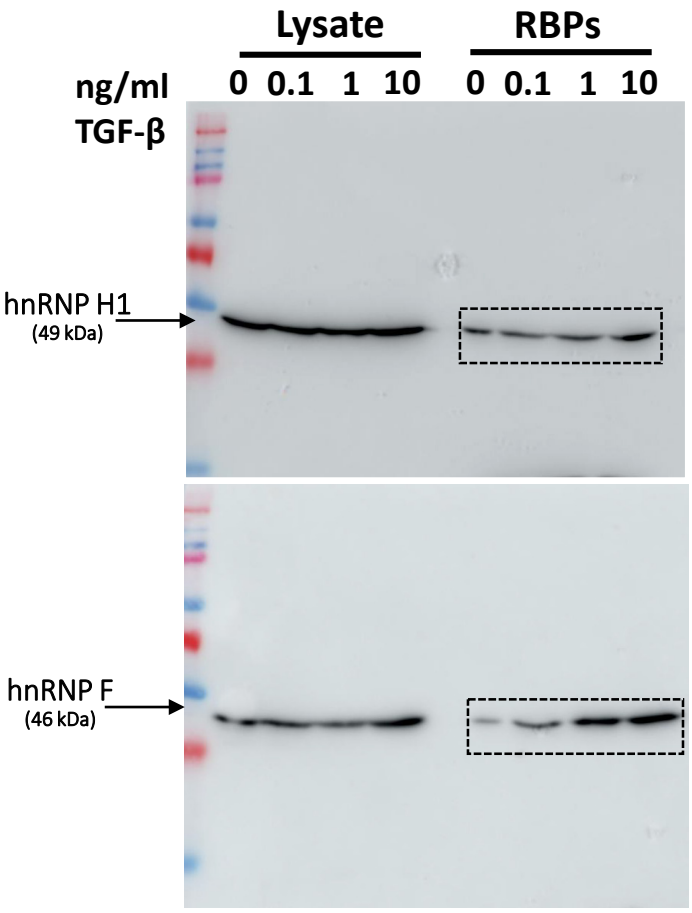
**Source data**

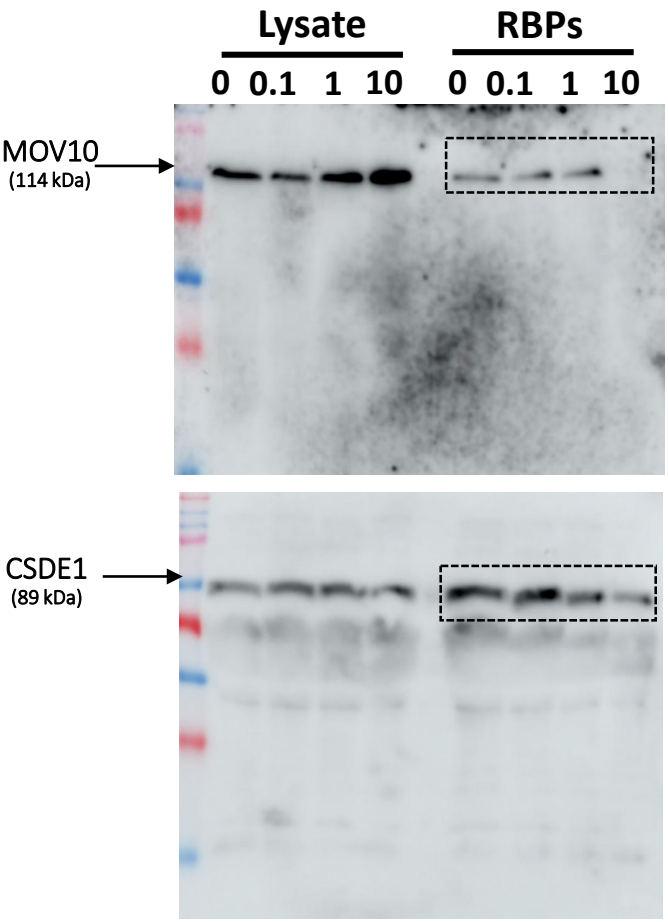
Source data related to Figure 1

Uncropped/unprocessed representative Western blots relating to Figure 1G. Boxes reflect data quantified in Figure 1 H (validation of TGF- β driven changes in cross-linked RBPs.)

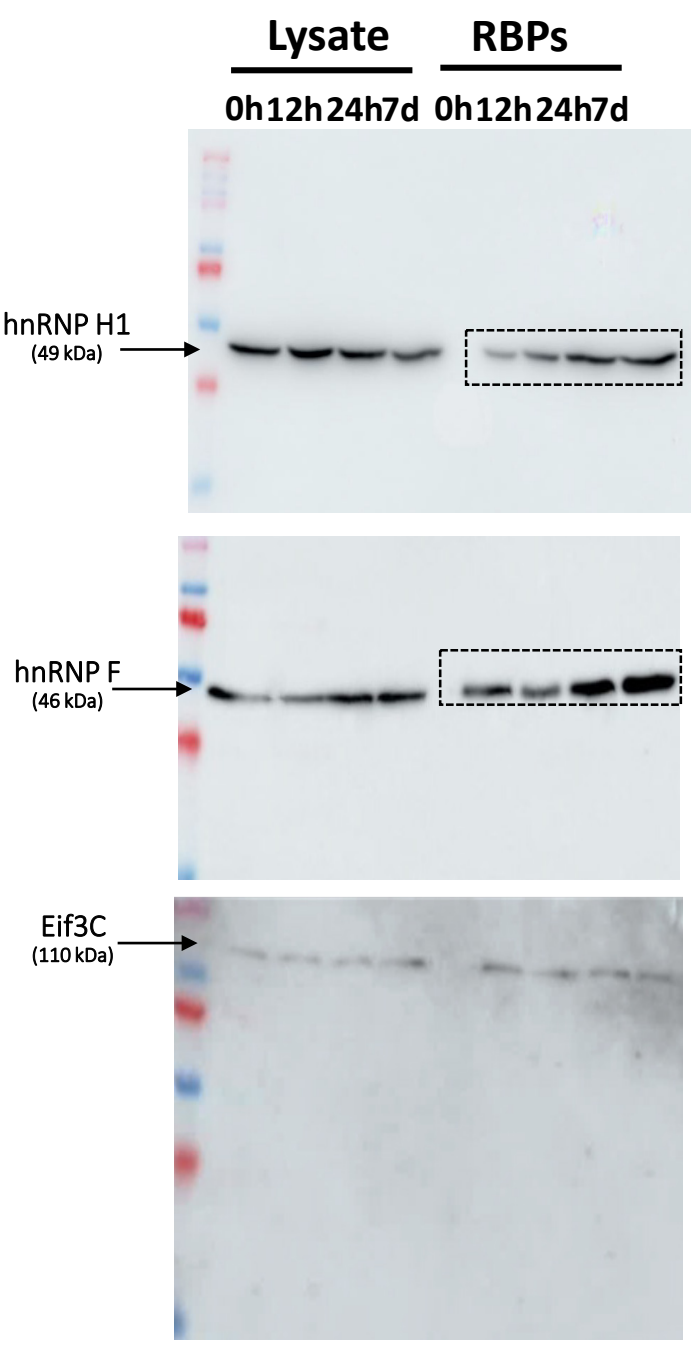


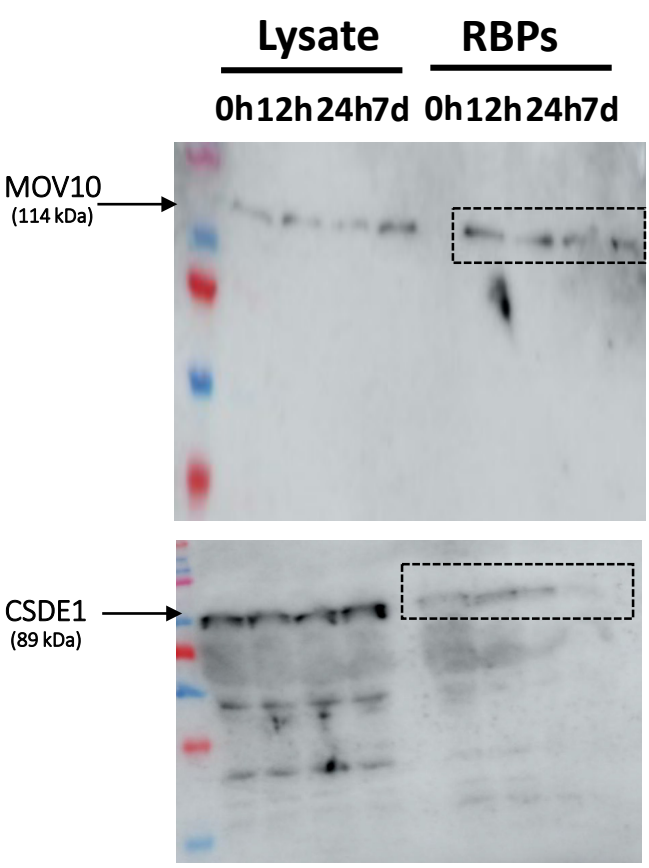
Source data relating to Figure 2.
Uncropped/unprocessed representative Western blots relating to Figure 2C. Boxes reflect data quantified in Figure 2D (Quantification of dose dependent TGF- β driven changes in RBPs.)



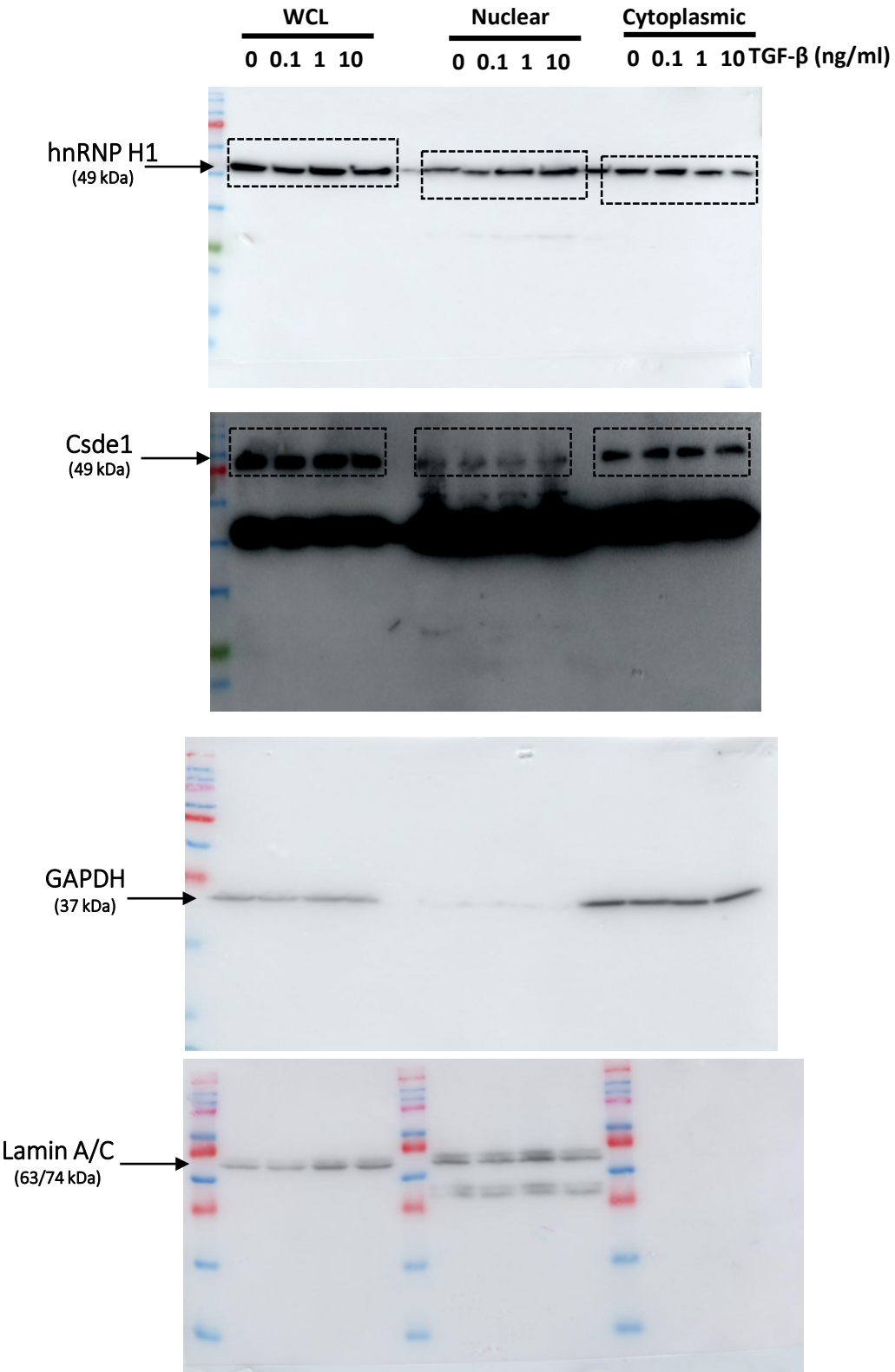



Uncropped/unprocessed representative Western blots relating to Figure 2E. Boxes reflect data quantified in Figure 2F (Quantification of time dependent TGF- β driven changes in RBPs.)

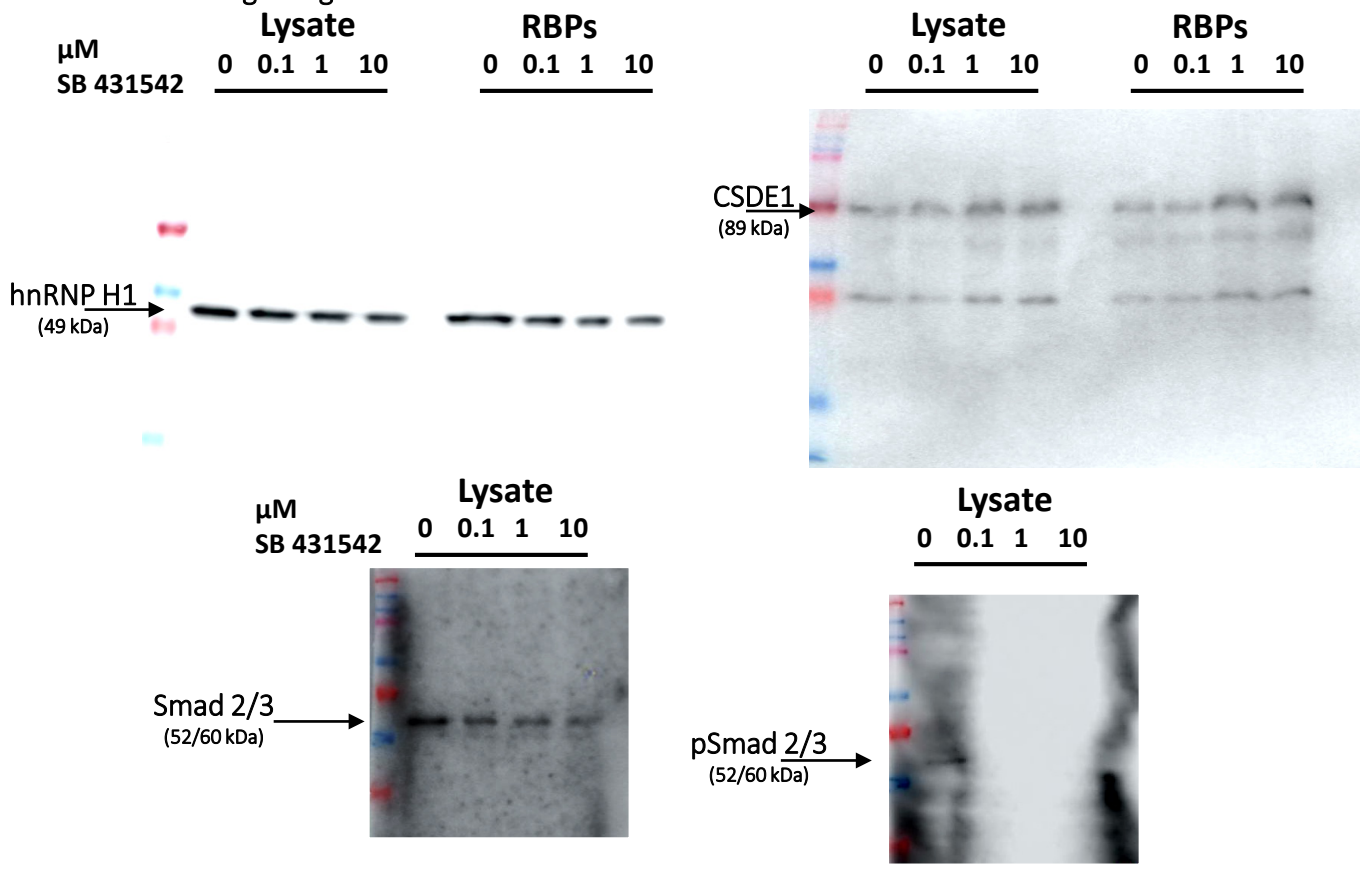


Uncropped/unprocessed representative Western blots relating to Figure 2G. Boxes reflect quantified data shown in H. Lamin A/C and Gapdh were used as markers to show efficient separation of the nuclear and cytoplasmic fractions.



Source data relating to Figure 2I.

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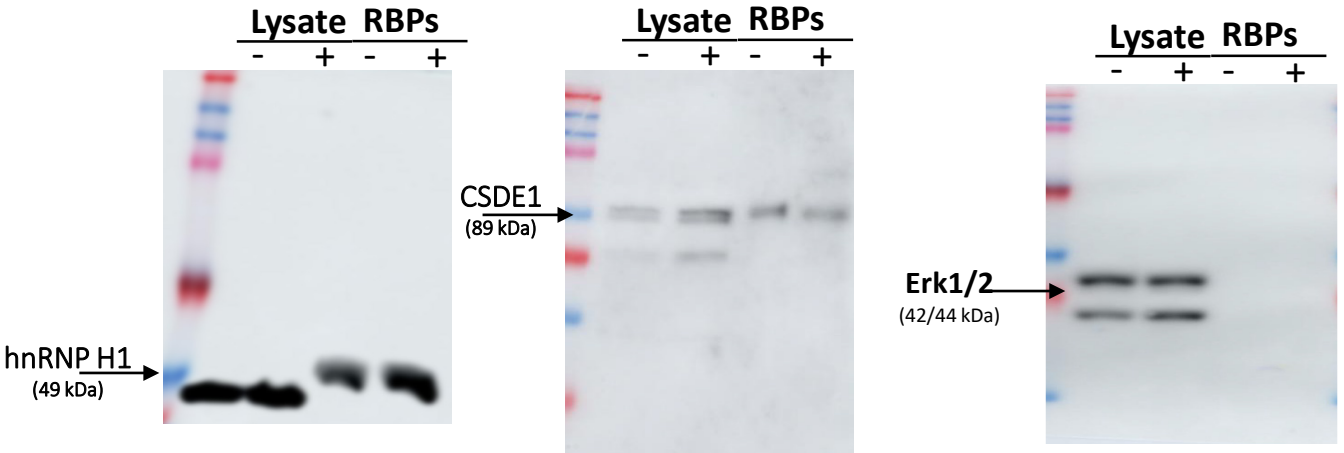


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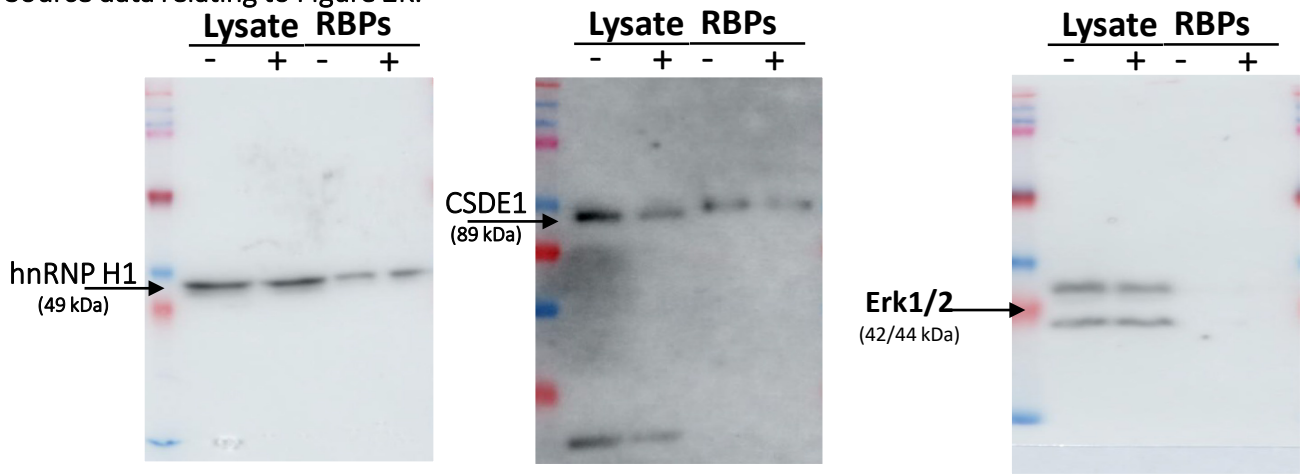
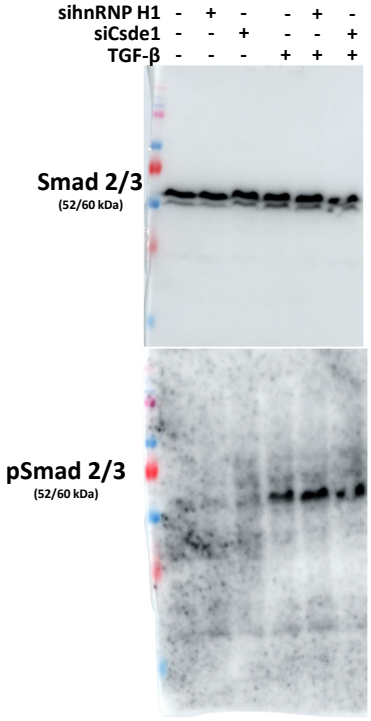


Source data related to Figure 3.

Source data related to Figure 3F.

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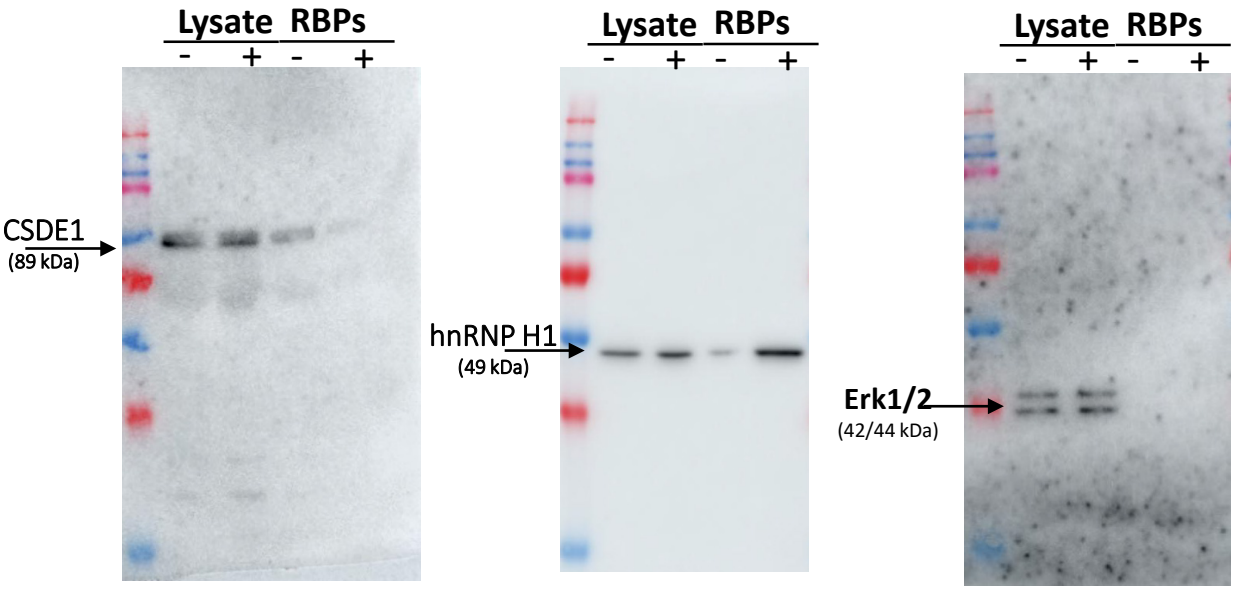
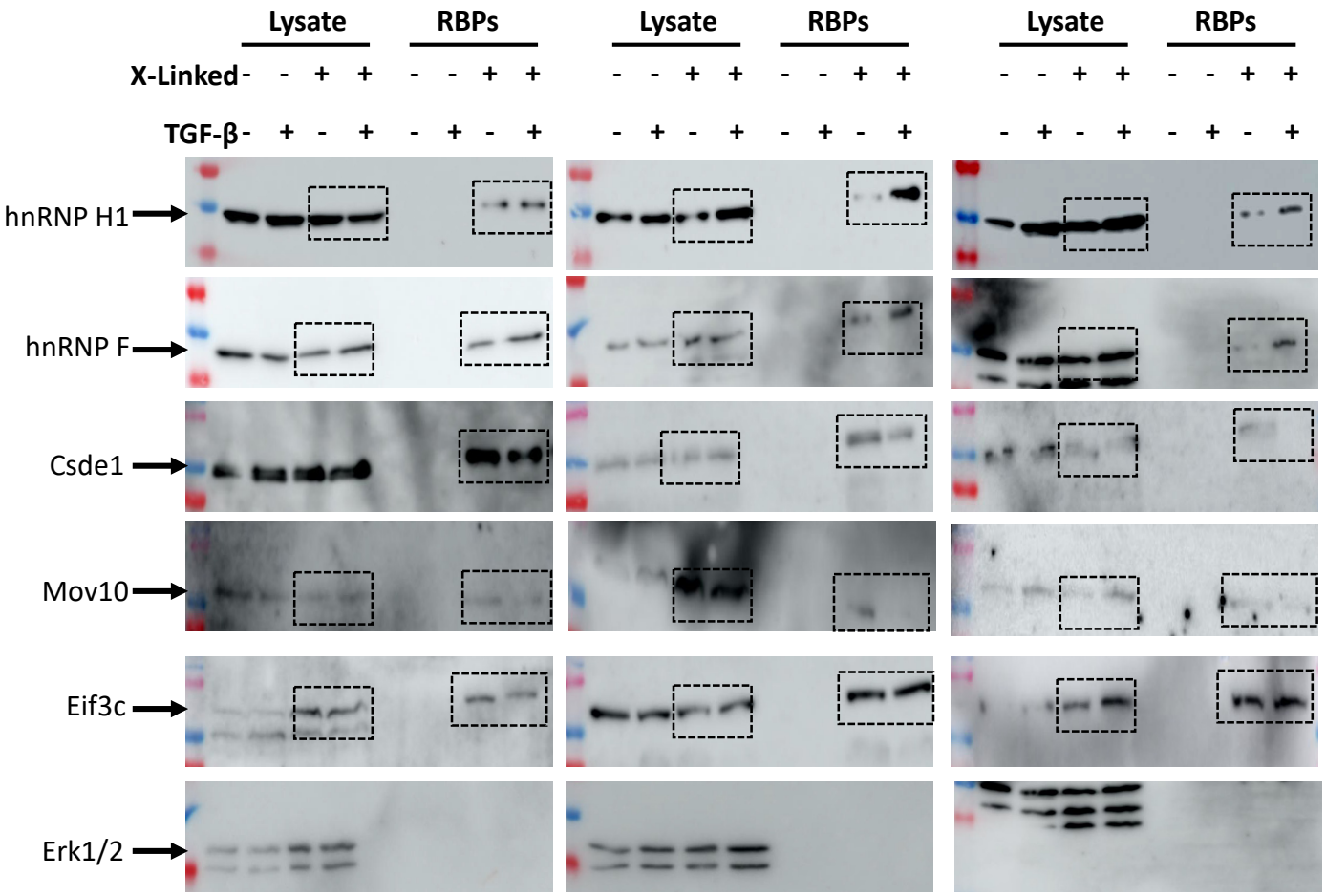
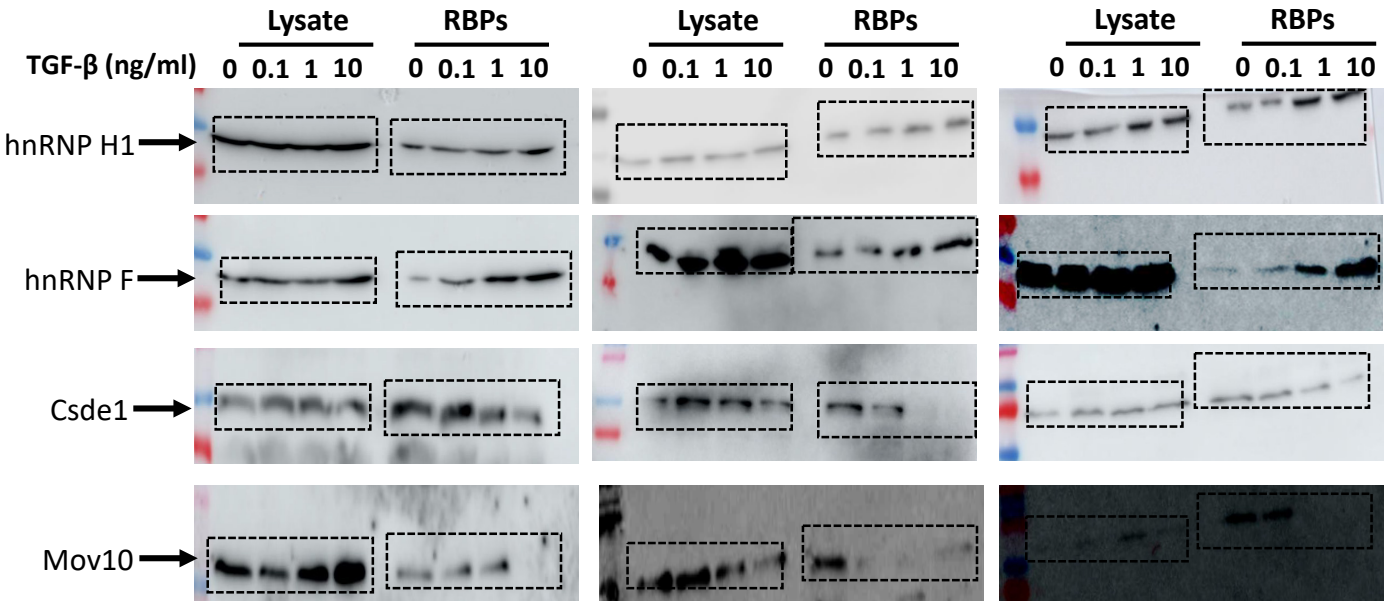


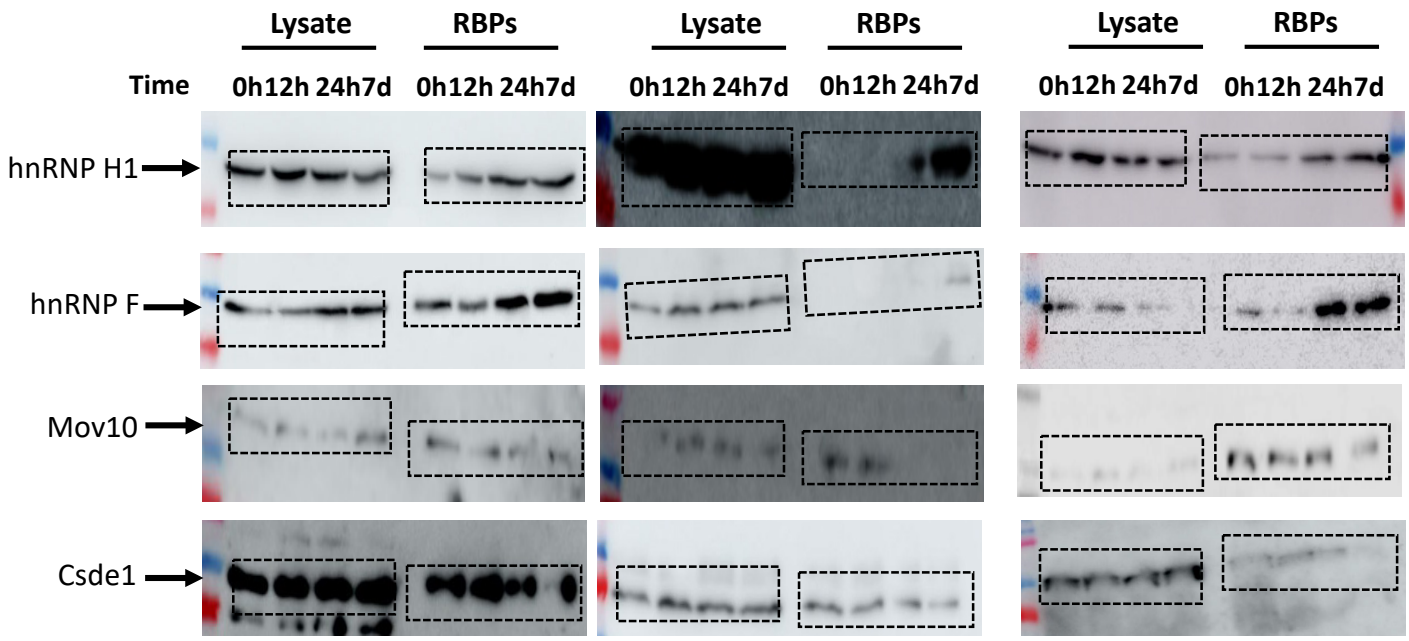
Full source data related to Figure 1G. Boxes reflect data quantified in Figure 1 H (validation of TGF- β driven changes in cross-linked RBPs relative to the corresponding input lysate)



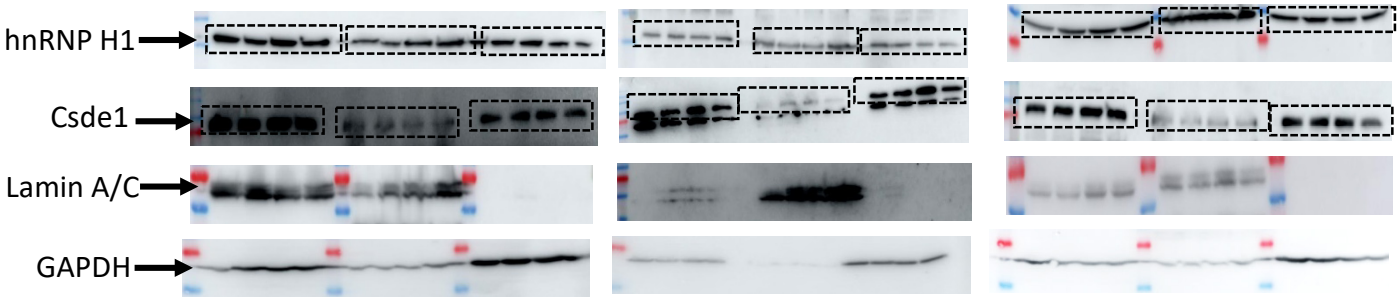
Full source data related to Figure 2C. Boxes reflect data quantified in Figure 2D (Quantification of dose dependent TGF- β driven changes in RBPs relative to lysate.)



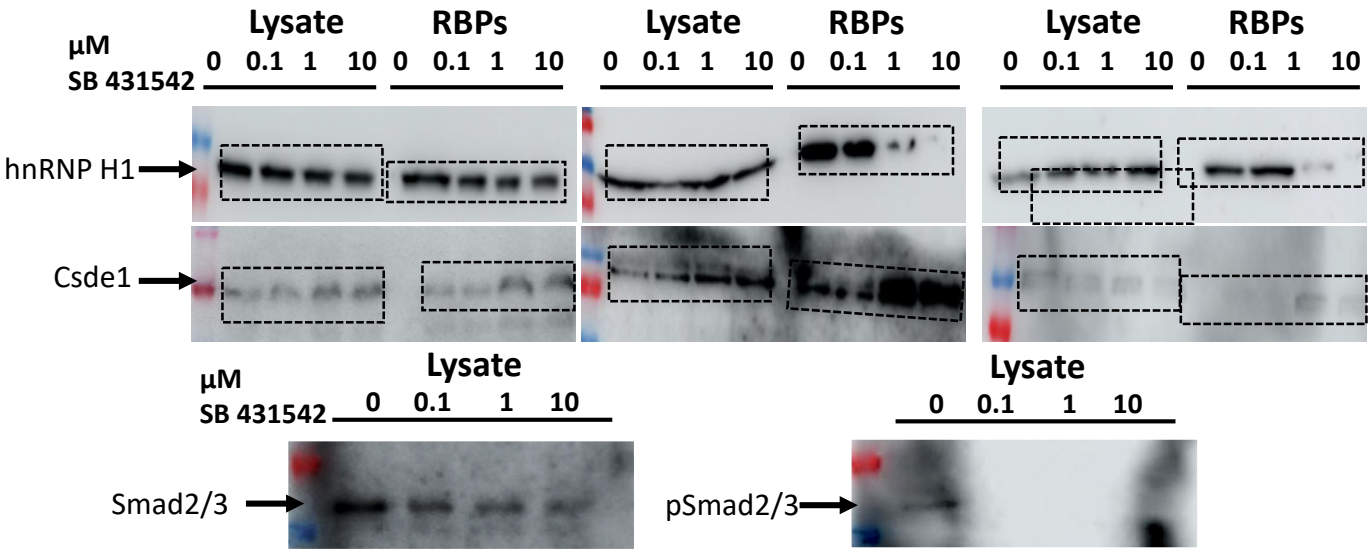
Full source data related to Figure 2E. Boxes reflect data quantified in Figure 2F (Quantification of time dependent TGF- β driven changes in RBPs relative to lysate .)



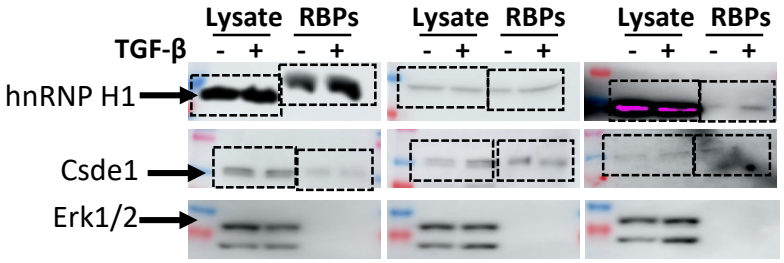
Full source data related to Figure 2G and H. Boxes reflect quantified data shown in H. Lamin A/C and Gapdh were used as markers to show efficient separation of the nuclear and cytoplasmic fractions.



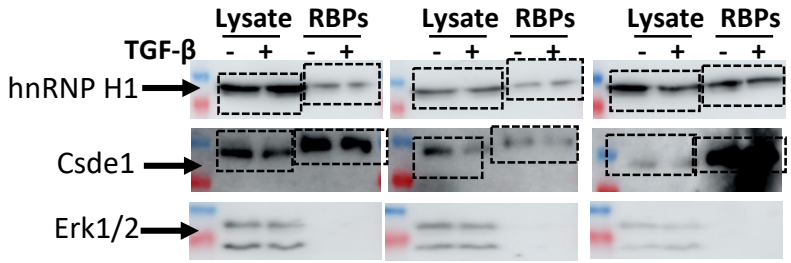
Full source data related to Figure 2I



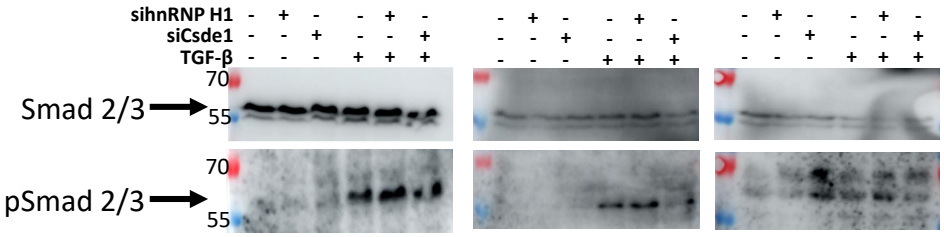
Full source data related to Figure 2J. Boxes reflect data shown in quantifications (changes in RNA binding in response to TGF-β).



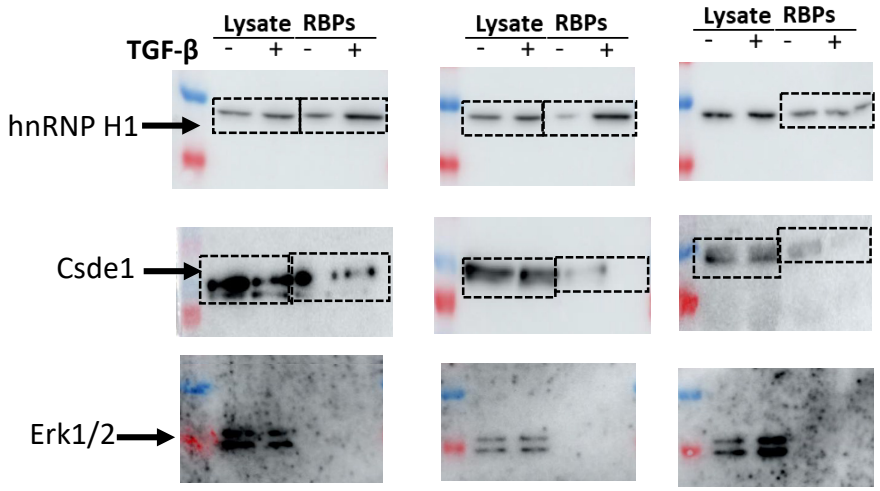

Full source data related to Figure 2K. Boxes reflect data shown in quantifications (changes in RNA binding in response to TGF-β).



Full source data related to Figure 3F




Full source data related to Figure 3G. Boxes reflect data shown in quantifications (changes in RNA binding in response to TGF-β).



# Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

## Animals (in vivo studies)

| Species | Vendor or Source | Background Strain | Sex | Persistent ID / URL |
|---|---|---|---|---|
| Mouse | Charles River | C57Bl6N | Male | https://www.criver.com/products-services/find-model/c57bl6-mouse?region=23 |

## Genetically Modified Animals

| | Species | Vendor or Source | Background Strain | Other Information | Persistent ID / URL |
|---|---|---|---|---|---|
| **Parent - Male** | N/A | | | | |
| **Parent - Female** | N/A | | | | |

## Antibodies

| Target antigen | Vendor or Source | Catalog # | Working concentration | Persistent ID / URL |
|---|---|---|---|---|
| hnRNP H1 | Thermo Fisher | PA5-50678 | 1:1000 (Western blot)<br>1:500 (PLA) | https://www.thermofisher.com/antibody/product/hnRNP-H1-Antibody-Polyclonal/PA5-50678 |
| hnRNP H1 | Thermo Fisher | PA5-70400 | 5 µg/mg (IP) | https://www.thermofisher.com/antibody/product/hnRNP-H1-Antibody-Polyclonal/PA5-70400 |
| CSDE1 | Thermo Fisher | PA5-96480 | 1:1000 (Western blot)<br>5 µg/mg (IP)<br>1:250 (PLA) | https://www.thermofisher.com/antibody/product/CSDE1-Antibody-Polyclonal/PA5-96480 |
| hnRNP F | Novus | NBP2-57442 | 1:1000 (Western blot) | https://www.novusbio.com/products/hnrnp-f-antibody_nbp2-57442 |
| Mov10 | Santa-Cruz | sc-515722 | 1:500 (Western blot) | https://www.scbt.com/p/mov10-antibody-b-3 |
| eIF3c | Thermo Fisher | PA5-17110 | 1:500 (Western blot) | https://www.thermofisher.com/antibody/product/eIF3c-Antibody-Polyclonal/PA5-17110 |
| GAPDH | Fitzgerald | 10R-G109a | 1:1000 (Western blot) | https://www.citeab.com/antibodies/10173-10r-g109a-gapdh-antibody |
| Lamin A/C | Cell Signaling | 2032 | 1:1000 (Western blot) | https://www.cellsignal.com/products/primary-antibodies/lamin-a-c-antibody/2032 |

DOI [to be added]

| | | | | |
|---|---|---|---|---|
| | Technologies | | | |
| Phospho-Smad2 (Ser465/467)/ mad3 (Ser423/425) | Cell Signaling Technologies | 8828S | 1:1000 (Western blot) | https://www.cellsignal.com/products/primary-antibodies/phospho-smad2-ser465-467-smad3-ser423-425-d27f4-rabbit-mab/8828?site-search-type=Products&N=4294956287&Ntt=8828s&fromPage=plp&_requestid=1540768 |
| Smad2/3 | Cell Signaling Technologies | 3102S | 1:1000 (Western blot) | https://www.cellsignal.com/products/primary-antibodies/smad2-3-antibody/3102 |
| ERK 1/2 | Cell Signaling Technologies | 9102 | 1:1000 (Western blot) | https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-antibody/9102 |
| GSL I - isolectin B4 antibody | Vector | FL-1201 | 1:50 (IF) | https://vectorlabs.com/products/glycobiology/fluorescein-gsl-i-isolectin-b4 |
| Biotin | Abcam | ab201341 | 1:500 (PLA) | https://www.abcam.com/products/primary-antibodies/biotin-antibody-hyb-8-ab201341.html |
| IgG control (rabbit) | Cell Signaling Technologies | 2729S | Experimentally matched | https://www.cellsignal.com/products/primary-antibodies/normal-rabbit-igg/2729 |

**DNA/cDNA Clones**

| Clone Name | Sequence | Source / Repository | Persistent ID / URL |
|---|---|---|---|
| Primers (5'-3') (Mouse unless stated otherwise) | | | |
| *Pecam1* | F-GGAAGTGTCCTCCCTTGAGC<br>R-GGAGCCTTCCGTTCTTAGGG | Sigma/Merck | This paper |
| *eNos* | F-TGGCATGGGCAACTTGAAGA<br>R-CGGTGCTGGAGAGGCTG | Sigma/Merck | This paper |
| *Fn1* | F-TGTGACAACTGCCGTAGACC<br>R-TGGGGTGTGGATTGACCTTG | Sigma/Merck | This paper |
| *α-Sma* | F-GAGACTCTCTTCCAGCCATCT<br>R-CCCTGACAGGACGTTGTTAGC | Sigma/Merck | This paper |
| *Sm22α* | F-GCCACACTGCACTACAATCC<br>R-CCAGTCCACAAACGACCAAG | Sigma/Merck | This paper |
| *hnRNP H1* | F- GCTTTTTGTGGAGCCCCG<br>R- TTCTGCTCCCAGCATCATCG | Sigma/Merck | This paper |
| *Csde1* | F- CATCCTTTGGAACTTGTGCTGA<br>R- TGGATCAAAGCTCATCTCGCA | Sigma/Merck | This paper |
| *Smad6* | F-TCCGGGTGAATTCTCAGATGC<br>R- GCCCTGAGGTAGGTCGTAGA | Sigma/Merck | This paper |
| *Col1a1* | F-CCGCTGGTCAAGATGGTC<br>R-CCTCGCTCTCCAGCCTTT | Sigma/Merck | This paper |
| *Itga3* | F-ACAGAGTCAGGGTAGATGGCT<br>R-AGAGGAGGATGATGAGCCCC | Sigma/Merck | This paper |

| | | | |
|---|---|---|---|
| *Col5a1* | F- GATCCCAACCAAGGGTGCTC<br>R- CCAAGAAGTGATTCTGGCTCCC | Sigma/Merck | This paper |
| *Col3a1* | F-AAGGCTGAAGGAAACAGCAA<br>R- TGGGGTTTCAGAGAGTTTGG | Sigma/Merck | This paper |
| *TGF-ß2* | F-CAGCGCTACATCGATAGCAA<br>R-CCTCGAGCTCTTCGCTTTTA | Sigma/Merk | This paper |
| *hnRNP H1 (human)* | F-<br>CAGTTCAGCGACCACGTTTG<br>R-<br>CACCACGAATCCCTCTCCAC | Sigma/Merck | This paper |
| *Csde1 (human)* | F- CGCTGAGCTGTTGGGTATGA<br>R- ACGAGGTTTGTTCCTTGCCT | Sigma/Merck | This paper |
| PLA Probes 5'-3' | | | |
| *Smad6* | 1.CTCAATCGGTGTTCGGAATGAA[BtnTg]<br>2.CACAGAGATCGTAGCAAAGCGA[BtnTg]<br>3.GAGGTAGTTCCACAAGCTGAAA[BtnTg]<br>4. AGGATGAGTTGTTGGTGTCT[BtnTg] | Sigma/Merck | This paper |
| *Col1a1* | 1.CGTTTCTCAGATGTACAGATCC[BtnTg]<br>2.GATCTGTACAAGTCGAAACACC[BtnTg]<br>3.GATACCGATACTACTTTTTAGT[BtnTg]<br>4.CTCAGTCGTCTAACTCCTGTAG[BtnTg] | Sigma/Merck | This paper |
| *Itga3* | 1.CATTTTTTAACGGACTGATGGC[BtnTg]<br>2.GTAAAGTCTCTTTTCACTGGGA[BtnTg]<br>3.AGAGGAAGTTCTGGAACGTTAC[BtnTg]<br>4.GATTACCGGACGAGACTATATA[BtnTg] | Sigma/Merck | This paper |
| *Col5a1* | 1.CGAAGGATACTGAGGGACTT[BtnTg]<br>2. CTAGAAGACCTCTACGATCT[BtnTg]<br>3. CATGTTTACTGGAAGGACGC[BtnTg]<br>4.ATAGGACGGAAAGGATGTCG[BtnTg] | Sigma/Merck | This paper |

**Cultured Cells**

| Name | Vendor or Source | Sex (F, M, or unknown) | Persistent ID / URL |
|---|---|---|---|
| Mouse cardiac endothelial cells (MCECs) | CELLultions biosystems inc. | unknown | CLU510 |
| Human umbilical vein endothelial cells (HUVECs) | PromoCell | unknown | C-12200 |
| NIH/3T3 | ATCC | unknown | CRL-1658 |
| HL-1 | Merck | Female | SCC065 |
| Human Cardiac Microvascular Endothelial Cells (HCMEC) | Promocell | unknown | C-12286 |

**Data & Code Availability**

| Description | Source / Repository | Persistent ID / URL |
|---|---|---|

DOI [to be added]

| | | |
|---|---|---|
| RIC data | Supplementary information | This paper |
| RIP-seq and RNA seq data | Supplementary information and the GEO repository | This paper and GSE216228 |

**Other**

| Description | Source / Repository | Persistent ID / URL |
|---|---|---|
| Key reagents | | |
| Oligo d(T)25 Magnetic beads | New England Biolabs | S1419S |
| Lithium chloride (LiCl) | Sigma/Merck | 203637 |
| Lithium dodecyl sulphate (LiDS) | Sigma/Merck | L9781 |
| EDTA | Sigma/Merck | 03609 |
| Dithiothreitol (DTT) | Sigma/Merck | 43819-5G |
| Tris | Carl Roth | 4855.3 |
| Tween 20 | Carl Roth | 9127.1 |
| IGEPAL CA-630 (NP-40) | Sigma/Merck | I8896 |
| Complete Mini EDTA free protease inhibitor cocktail | Roche | 4693159001 |
| RNase A/T1 Mix | Thermo Fisher | EN0551 |
| DMEM (high glucose, 4.5 g/L) | Pan biotech | P04-03500 |
| Feotal calf serum (FCS) | Merck/Millipore (Biochrom) | S0615 |
| Penicilin-streptomycin | Thermo/Life Technologies | 15140122 |
| 1M HEPES | Thermo/Life Technologies | 15630056 |
| MEM Non-Essential Amino Acids Solution (100X) | Thermo/Life Technologies | 11140050 |
| PBS | Pan biotech | P04-53500 |
| Trypsin, 2.5% | Thermo/Life Technologies | 15090046 |
| Endothelial cell growth media kit | Promocell | C-22110 |
| Lipofectamine RNAiMAX transfection reagent | Thermo Fisher Scientific | 13778150 |

| | | |
|---|---|---|
| Corning Matrigel basement membrane matrix | Corning | 354234 |
| Hnrnph1, Mouse, ORF Clone (GFP tagged) | Origene | MG207170 |
| Csde1, Mouse, ORF clone (untagged) | Origene | MC202422 |
| Lipofectamine 3000 Transfection reagent | Thermo Fisher Scientific | L3000-015 |
| Vectashield HardSet Mounting Medium with DAPI | Vector | VEC-H-1500 |
| Recombinant TGF-β1 (HEK-293T derived) | Peprotech | 100-21 |
| Surebeads, Protein A | BioRad | 1614013 |
| RNaseIN Ribonuclease Inhibitor | Promega | N2515 |
| ProSieve™ QuadColor™ Protein Marker | Lonza | 00193837 |
| Prestained protein marker | Proteintech | PL00001 |
| $MgCl_2$ | Carl Roth | KK36.3 |
| NaCl | Carl Roth | 9265.2 |
| SDS | Carl Roth | 2326.3 |
| Proteinase K | New England Biolabs | P8107S |
| Phenol chloroform | Sigma/Merck | 77617 |
| Phase Lock Heavy tube | Avantor | 733-2478 |
| TURBO DNase | Thermo Fisher Scientific | AM2238 |
| Pictilisib (GDC-0941) | MedChemExpress | HY-50094 |
| PD 0325901 | Sigma/Merck | PZ0162 |
| Triton x 100 | Carl Roth | 3051.3 |

DOI [to be added]

| | | |
|---|---|---|
| Bovine Serum Albumin Fraction V | Sigma/Merck | 10735108001 |
| Triethanolamine | Carl Roth | 6300.1 |
| Acetic anhydride | Sigma/Merck | 320102 |
| SSC buffer (20x) | Sigma/Merck | S6639 |
| Denhardts reagent | Thermo Fisher Scientific | 750018 |
| CHAPS | Carl Roth | 1479.1 |
| tRNA | Sigma/Merck | 10109495001 |
| Heparin sodium salt | Sigma/Merck | H3393 |
| Deionised formamide | Sigma/Merck | F9037 |
| Actinomycin D | Sigma/Merck | A9415-5MG |
| Collagenase I | Worthington biochem | LS004176 |
| DNase I | Worthington biochem | LS002139 |
| RPMI 1640 | Thermo/Life Technologies | 31870025 |
| MACS buffer | Miltenyi | 130091222 |
| CD146 microbeads | Miltenyi | 130092007 |
| 0.1% BCECF AM Ester | Sigma/Merck | B8806 |
| Kits and assays | | |
| Maxima H Minus First Strand cDNA Synthesis Kit | Thermo Fisher Scientific | K1652 |
| NucleoSpin RNA Extraction kit | Macherey-Nagel | 740955 |
| Maxima SYBR Green qPCR Master Mix | ThermoFisher Scientific | K0253 |
| Cell based LDL-uptake assay kit | Abcam | ab133127 |
| Zymo RNA clean and concentrator kit | Zymo | R1015 |
| Agilent High Sensitivity DNA Kit | Agilent Technologies | 5067-4626 |
| Duolink *In Situ* Red Starter Kit Mouse/Rabbit | Sigma/Merck | DUO92101 |
| Oligonucleotides | | |
| ON-TARGETplus siRNA, hnRNP | Dharmacon/horizon discovery | LQ-048699-01-0010 |

DOI [to be added]

| | | |
|---|---|---|
| H1 (mouse), set of 4 | | |
| ON-TARGETplus siRNA, Csde1 (mouse), set of 4 | Dharmacon/horizon discovery | LQ-040691-01-0010 |
| ON-TARGETplus siRNA, hnRNP H1 (human), set of 4 | Dharmacon/horizon discovery | L-012107-00-0010 |
| ON-TARGETplus siRNA, hnRNP H1 (mouse), set of 4 | Dharmacon/horizon discovery | L-015834-00-0010 |
| Silencer Negative Control siRNA | Thermo Fisher | AM4635 |
| Software and algorithms | | |
| IsobarQuant | https://www.bioconductor.org/packages/release/bioc/html/isobar.html | Version 3.17 |
| Limma | https://bioconductor.org/packages/release/bioc/html/limma.html | Version 3.5 |
| Vsn | https://www.bioconductor.org/packages/release/bioc/html/vsn.html | Version 3.62 |
| Msnbase | https://bioconductor.org/packages/release/bioc/html/MSnbase.html | Version 2.20.4 |
| CASAVA | Illumina | Version 1.8 |
| SAMBLASTER | https://github.com/GregoryFaust/samblaster | Version 0.1.26 |
| skewer | https://sourceforge.net/projects/skewer/files/Binaries/ | Version 0.1.126 |
| fastqc | https://qubeshub.org/resources/fastqc | Version 1.0 |
| Bowtie | http://bowtie-bio.sourceforge.net/index.shtml | Version 2.0 |
| BWA | http://bio-bwa.sourceforge.net/ | Version 0.7.12 |
| MACS2 | https://pypi.org/project/MACS2/ | Version 2.1.0 |
| Meme | https://meme-suite.org/meme/ | Version 4.10.2 |
| diffbind | https://bioconductor.org/packages/release/bioc/html/DiffBind.html | Version 3.4.11 |
| KOBAS | http://kobas.cbi.pku.edu.cn/kobas3/help/ | Version 3.0 |
| GOSeq | https://bioconductor.org/packages/release/bioc/html/goseq.html | Version 1.46 |
| HISAT2 | http://daehwankimlab.github.io/hisat2/ | Version 2.2.1 |
| featureCounts | https://rdrr.io/bioc/Rsubread/man/featureCounts.html | Version 2.4.3 |
| rMATs | http://rnaseq-mats.sourceforge.net/ | Version 4.1.0 |
| GraphPad Prism | https://www.graphpad.com/scientific-software/prism/ | Version 7.04 |
| ImageJ (Fiji) | https://imagej.net/software/fiji/downloads | |
| Stellaris Probe designer | https://www.biosearchtech.com/support/tools/design-software/stellaris-probe-designer | |

**ARRIVE GUIDELINES**

The ARRIVE guidelines (https://arriveguidelines.org/) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

**Study Design**

| Groups | Sex | Age | Number (prior to experiment) | Number (after termination) | Littermates (Yes/No) | Other description |
|---|---|---|---|---|---|---|
| Group 1 (Control) Sham (two weeks) | Male | 8-10 weeks at surgery | 3 | 3 | No | |
| Group 2 TAC (two weeks) | Male | 8-10 weeks at surgery | 3 | 3 | No | |

**Sample Size:** Please explain how the sample size was decided Please provide details of any a *prior* sample size calculation, if done.

**Inclusion Criteria**

Matched aged and sex.

**Exclusion Criteria**

Matched aged and sex.

**Randomization**

No randomisation.

**Blinding**

Unblind.

DOI [to be added]