SUPPLEMENTARY MATERIALS

Alternative splicing regulation of cell cycle genes by SPF45/SR140/CHERP complex controls cell proliferation

Elena Martín[1-2], Claudia Vivori[1-2 *], Malgorzata Rogalska[1]

Jorge Herrero[1-2] and Juan Valcárcel[1-3]

[1] Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr Aiguader 88, Barcelona 08003, Spain

[2] Universitat Pompeu Fabra (UPF), Barcelona 08003, Spain

[3] Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain

* Current address: The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK

MARTIN ET AL. SUPPLEMENTARY FIGURE LEGENDS

Figure S1 – Related to figure 1. DHX15 is distinctively linked to SPF45, SR140 and CHERP. (A) Splicing perturbation profiles upon SPF45, SR140, CHERP or DHX15 knockdowns as in figure 1A. (B-C) Validation of AS events from the perturbation profiles in A assessed by RT-PCR followed by capillary electrophoresis. PSI (Percent Spliced In) values are presented upon SPF45, SR140, CHERP or DHX15 knockdowns in HeLa and U2OS cells. Data are represented as mean ± SD of three biological replicates and p-values are calculated comparing to siCNT by Student's t-test (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$). (D) Western blot analyses of SPF45, SR140 and CHERP (and GAPDH as loading control) upon their individual siRNA-mediated knockdowns in HeLa cells. The results (equivalent to those of Figure 1B) are representative of three independent experiments.

Figure S2 – Related to figure 3. The SPF45/SR140/CHERP complex is required for cell proliferation, cell cycle progression and inhibition of apoptosis. (A) Same as in figure 3A using a second set of shRNAs (#2). (B) Same as in figure 3B using shRNAs #2. (C) Same as in figure 3C using shRNAs #2. The U2OS experiment was performed in biological duplicates. (D) Same as in figure 3D using shRNAs #2.

Figure S3 - Related to figure 3. Effects of SPF45 / SR140 / CHERP knock down on apoptosis and cell cycle. (A) Proportion of viable, early apoptotic and late apoptotic/necrotic HeLa or U2OS cells after lentivirus-mediated knockdown of SPF45, SR140 or CHERP measured by PI and annexin V-FITC staining and flow cytometry analyses. Data are represented as mean ± SD of three biological replicates with p-values calculated comparing to shCNT by one-way ANOVA followed by Dunnett's multiple comparison test (* $p < 0,01$; ** $p < 0,001$). See also figures S2-S3. (B) Flow cytometry analysis of HeLa cells stained with propidium iodide and phospho-H3 serine 10 antibody to discriminate between G2 and mitotic cells.

Figure S4 - Related to figure 3. Changes in expression of genes with functions related to extracellular space and plasma membrane upon lentivirus-mediated knockdown of SPF45, SR140 or CHERP. (A) Pie charts of differentially expressed genes upon SPF45, SR140 or CHERP knockdown in HeLa cells. Total RNA from duplicates of each condition was used for RNA-seq analysis and differential gene expression (log2 fold change ≥ 0,6 or ≤ -0,6) was quantified using Cuffdiff. (B) Venn diagram of the overlap of differentially expressed genes upon knockdown of SPF45, SR140 and CHERP. (C) GO analyses of differentially expressed genes upon knockdown of SPF45, SR140 or CHERP in HeLa cells performed using GOrilla. (D) Venn diagrams of the overlaps between alternatively spliced (AS) and differentially expressed genes (GE) upon knockdown of SPF45, SR140 or CHERP.

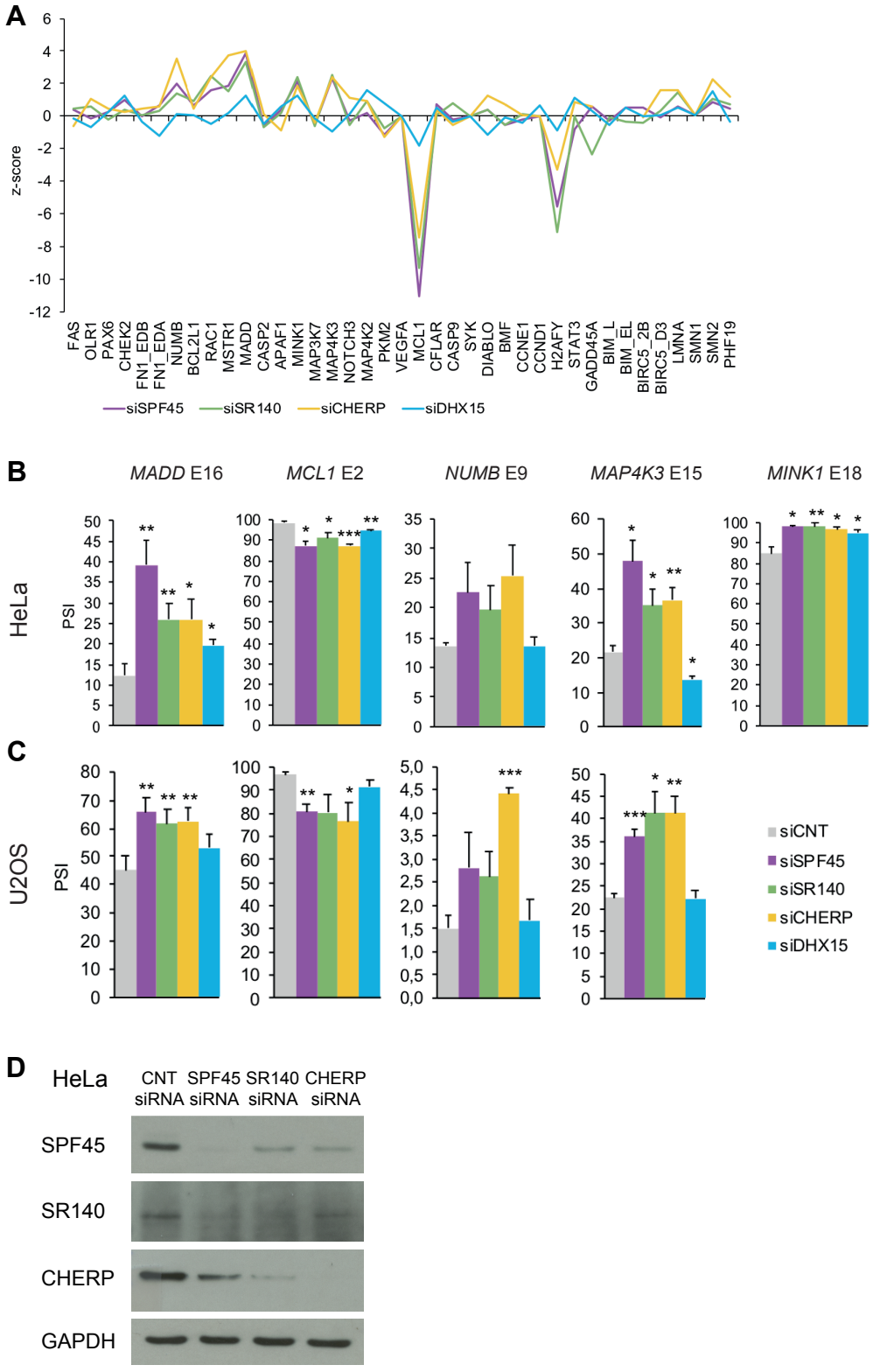
Figure S5 – Related to figure 4. SPF45, SR140 and CHERP co-regulate a program of AS events. (A-B) Validation of events detected by VAST-TOOLS by RT-PCR followed by capillary electrophoresis using two sets of shRNAs (#1 and #2) to knockdown SPF45, SR140 or CHERP in HeLa and U2OS cells. Data are represented as mean ± SD of three biological replicates and p-values are calculated relative to shCNT by Student's t-test (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$). (C) Venn diagrams of the overlap of all alternatively spliced events, alternative 3'ss, alternative 5'ss and RI between knockdown of SPF45, SR140 and CHERP according to VAST-TOOLS. (D) Summary of the number of CEx (y axis) changing towards inclusion (ΔPSI ≥ 15%) or towards skipping (ΔPSI ≤ -15%) in at least two of the knockdowns of SPF45, SR140 and CHERP, displayed as double overlaps (colored bars inside grey bars). (E) Distribution of exon lengths (y axis) for alternative exons more included (ΔPSI ≥ 15%, up) or more skipped (ΔPSI ≤ -15%, down) upon the knockdown of the three factors (triple overlap) compared to non-changing alternative exons. (F) Matt analysis of the length of introns flanking exons differentially spliced upon the knockdown of SPF45, SR140, CHERP or all three (triple overlap) compared to non-changing alternative exons. The statistical significance of the differences in the median length between each condition and non-

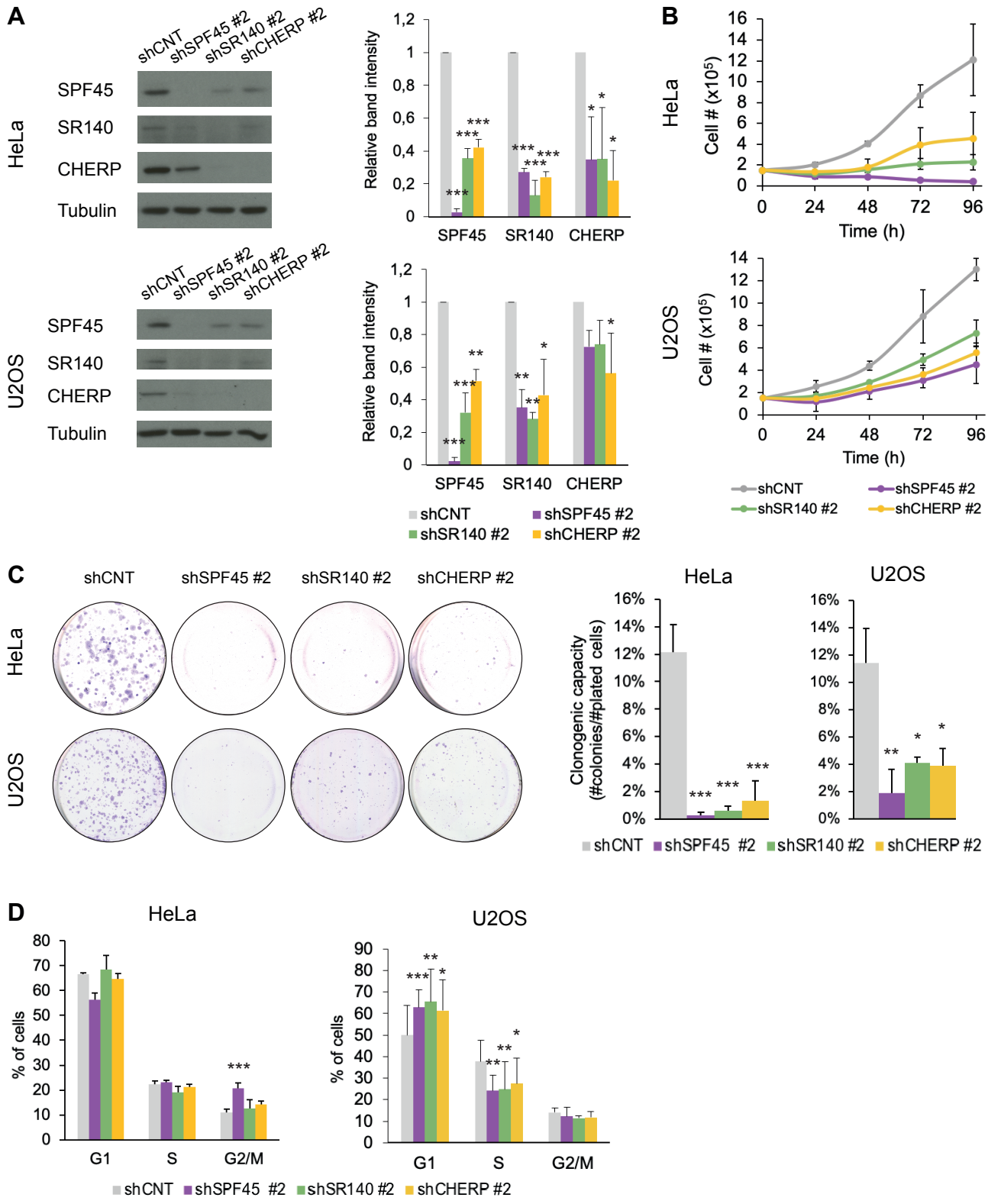
changing alternative exons was assessed using Mann-Whitney U-tests (* p < 0,05; ** p < 0,01; *** p < 0,001).

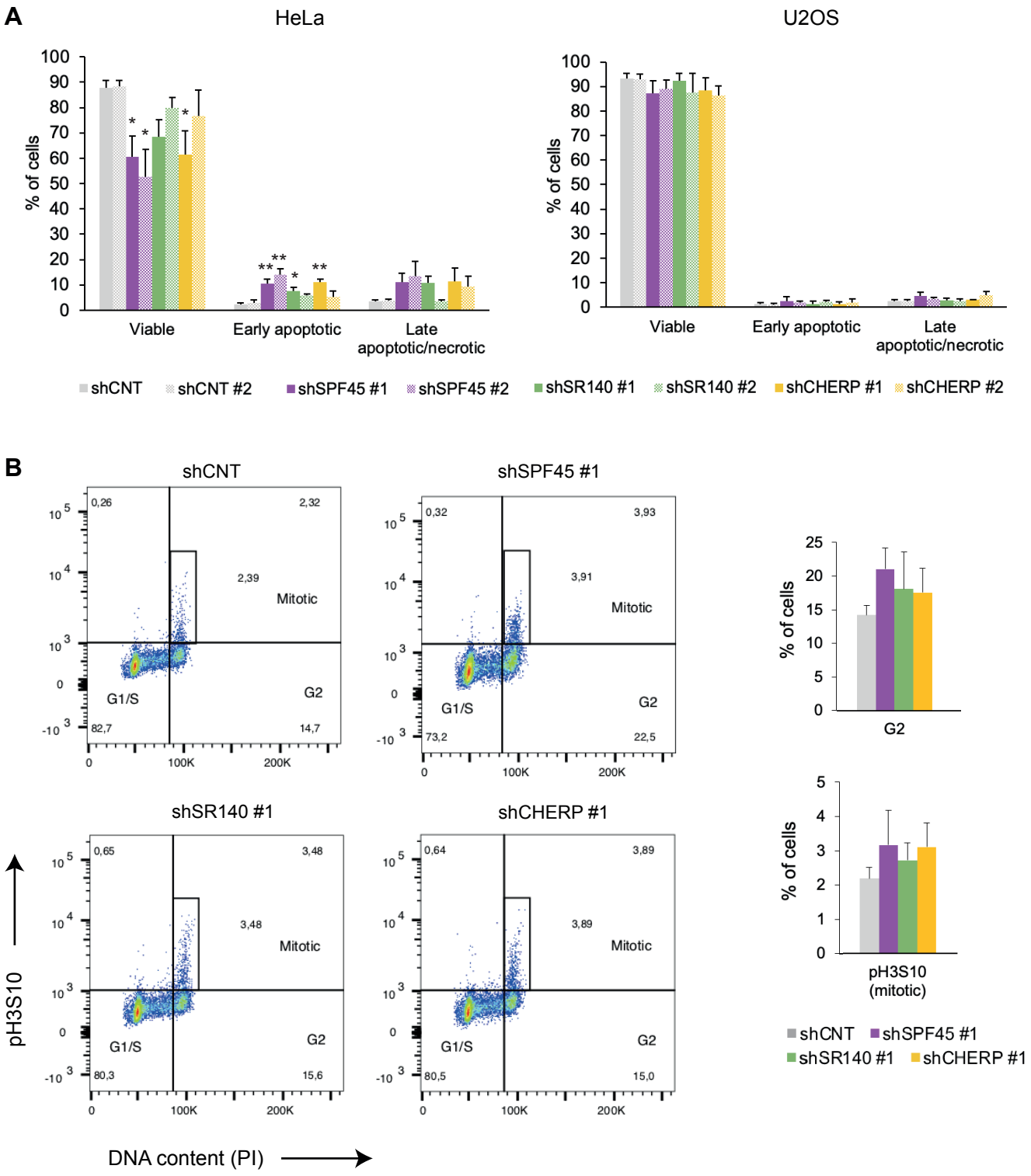
Figure S6 – Related to figure 4. Features of introns modulated upon knockdown of SPF45, SR140 or CHERP. (A) Matt analyses of splicing regulatory sequence features, GC content and length of more retained (ΔPSI ≥ 15%) and more spliced (ΔPSI ≤ -15%) introns upon knockdown of SPF45, SR140, CHERP or all three (triple overlap) compared to a stratified control set of non-changing introns. Sequence features analyzed include 5'ss and 3'ss strengths (maximum entropy scores), best predicted BP sequence score and Py sequence strength and length. The statistical significance of the normalized differences in the median of the scores of each feature between each condition and non-changing introns was assessed using Mann-Whitney U-tests (* p < 0,05; ** p < 0,01; *** p < 0,001). (B) Intron length (y axis) of retained (ΔPSI ≥ 15%) and spliced (ΔPSI ≤ -15%) introns upon knockdown of the three factors (triple overlap) compared to non-changing introns. (C-D) GO analyses of alternative exons differentially spliced upon knockdown of SR140 or CHERP in HeLa cells using GOrilla. (E) Prediction of the impact on the corresponding coding sequences of alternative exons more included upon knockdown of SPF45, SR140 or CHERP compared to the background exons. For each condition and background, the percentage of events (y axis) disrupting or preserving the coding sequence (CDS) (or not confidently predicted -CDS uncertain-) upon exon inclusion or skipping, affecting 3'UTR, 5'UTR or a non-coding RNA, are indicated.

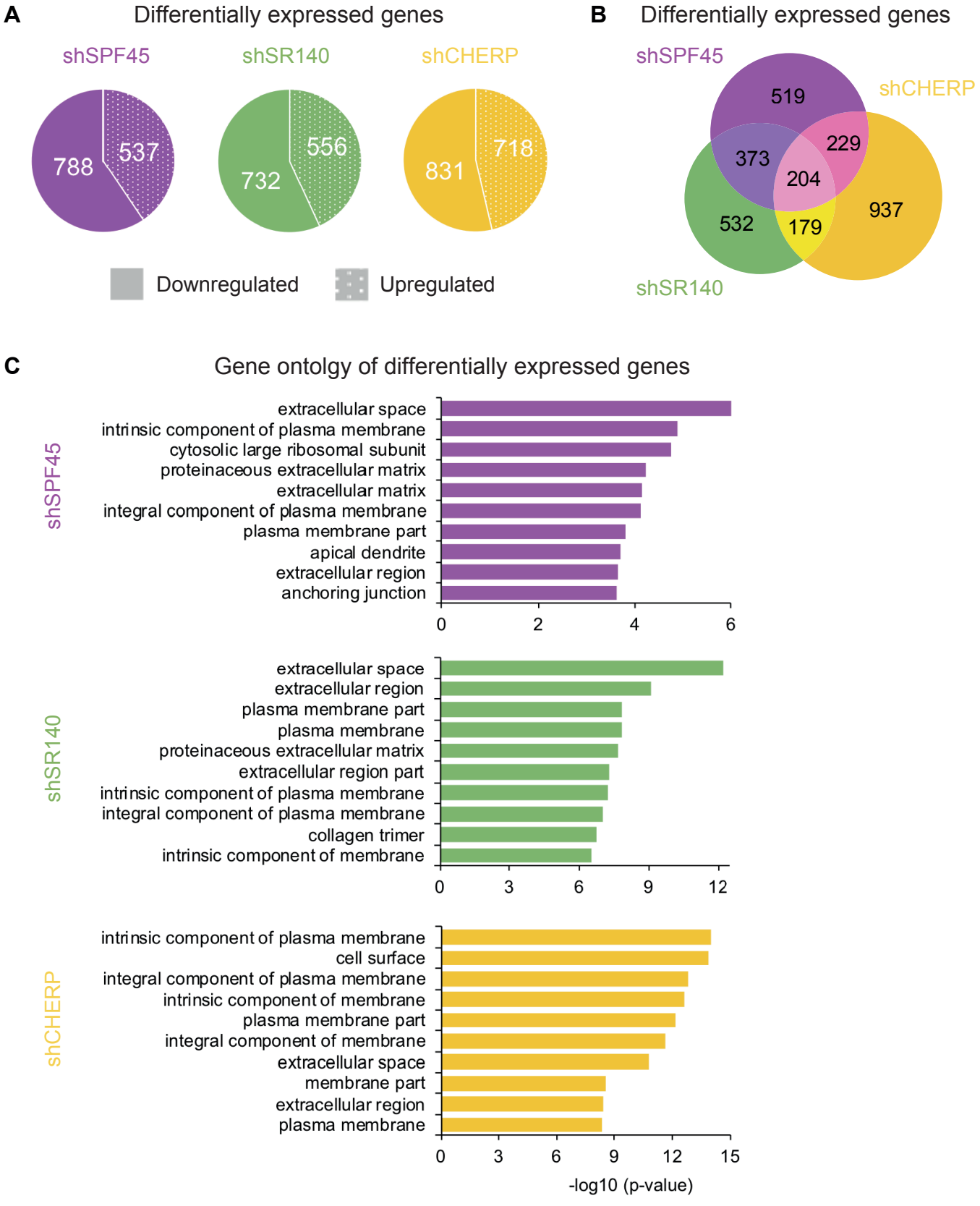
Figure S7 – Related to figure 5. SPF45/SR140/CHERP regulate FOXM1 AS. (A) Same as figure 5B using a second set of shRNAs (#2). (B) Quantification of FOXM1 exon 6 by RT-PCR followed by capillary electrophoresis upon knockdown of SPF45, SR140 or CHERP using shRNAs #1 and #2 in HeLa and U2OS cells. Data are represented as mean ± SD of three biological replicates and p-values are calculated comparing to shCNT by Student's t-test (* p < 0,05; ** p < 0,01). (C-D) Western blot analyses of FOXM1 protein levels (and tubulin as loading control), in HeLa and U2OS

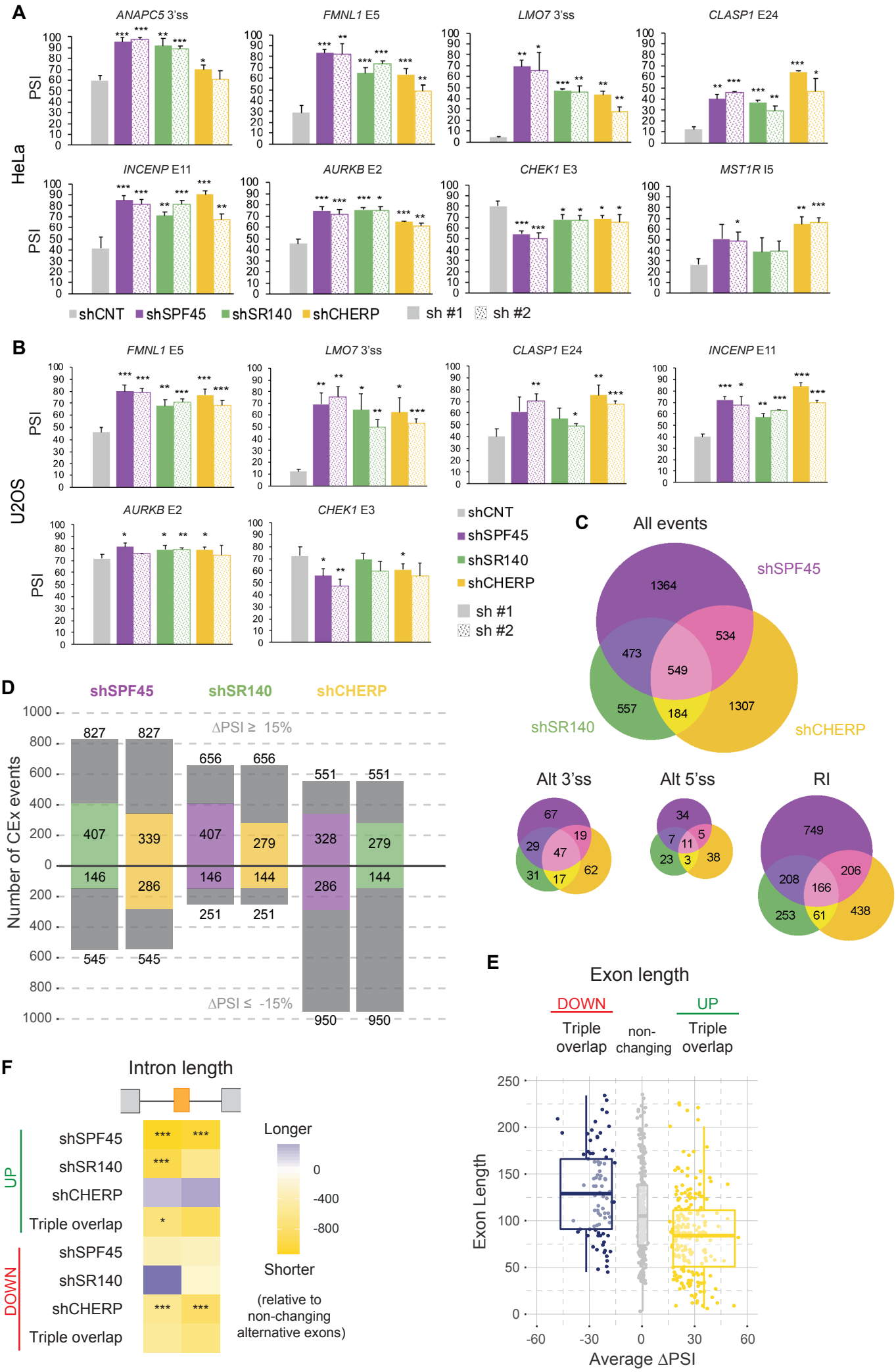
cells upon knockdown of SPF45, SR140 or CHERP with shRNAs #1 or #2 series. Right panel: quantification of relative band intensities using ImageJ. Data are represented as mean ± SD of three biological replicates with p-values calculated comparing to shCNT by one-way ANOVA followed by Dunnett's multiple comparison test (none are significant). (E) Expression fold change over shCNT of two FOXM1 targets upon knockdown of SPF45 and rescue of FOXM1 AS changes using AONs targeting exon 9 (experiments shown in Figures 6 B-C) measured by RT-qPCR. Data are represented as mean ± SD of three biological replicates and p-values are calculated by one-way ANOVA followed by Dunnett's multiple comparison test on ΔΔCt values (none are significant).

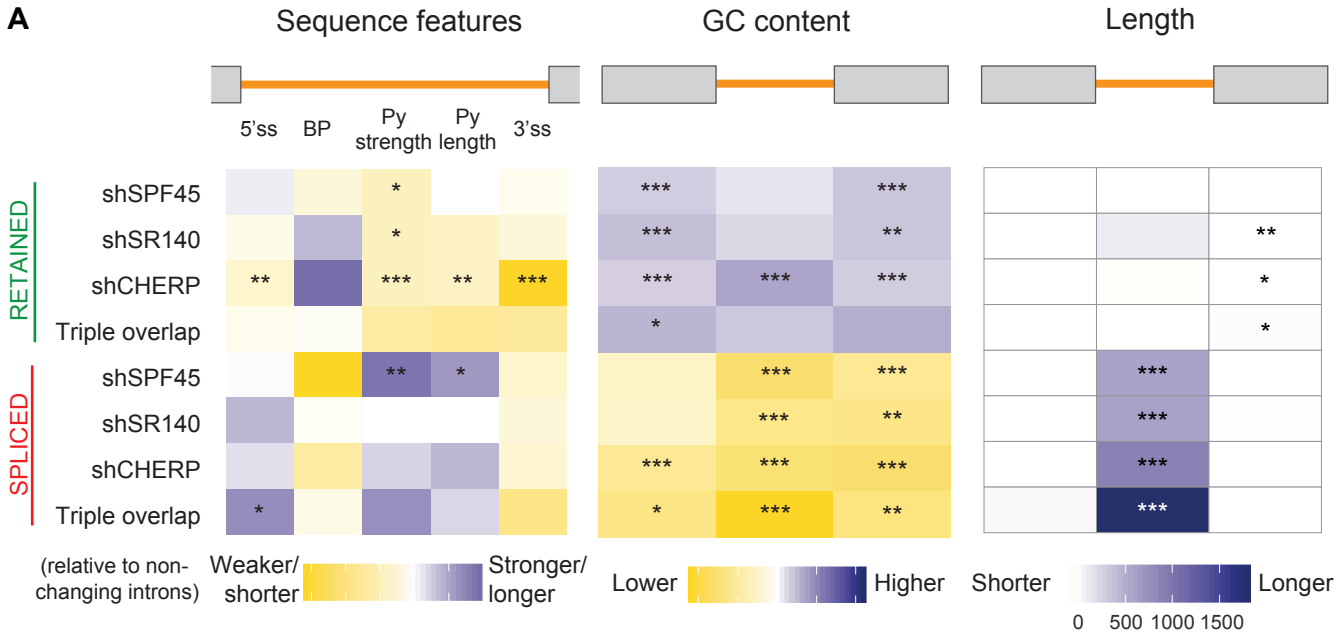

A
z-score
FAS
OLR1
PAX6
CHEK2
FN1_EDB
FN1_EDA
NUMB
BCL2L1
RAC1
MSTR1
MADD
CASP2
APAF1
MINK1
MAP3K7
MAP4K3
NOTCH3
MAP4K2
PKM2
VEGFA
MCL1
CFLAR
CASP9
SYK
DIABLO
BMF
CCNE1
CCND1
H2AFY
STAT3
GADD45A
BIM_L
BIM_EL
BIRC5_2B
BIRC5_D3
LMNA
SMN1
SMN2
PHF19
siSPF45
siSR140
siCHERP
siDHX15
B
MADD E16
MCL1 E2
NUMB E9
MAP4K3 E15
MINK1 E18
HeLa
PSI
C
U2OS
PSI
siCNT
siSPF45
siSR140
siCHERP
siDHX15
D
HeLa
CNT siRNA
SPF45 siRNA
SR140 siRNA
CHERP siRNA
SPF45
SR140
CHERP
GAPDH

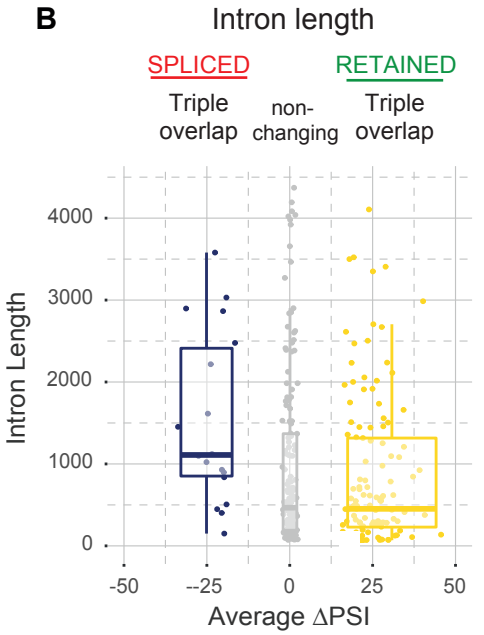

A
HeLa
shCNT
shSPF45 #2
shSR140 #2
shCHERP #2
SPF45
SR140
CHERP
Tubulin
U2OS
Relative band intensity
SPF45
SR140
CHERP
B
Cell # (x10⁵)
Time (h)
C
Clonogenic capacity (#colonies/#plated cells)
D
% of cells
G1
S
G2/M

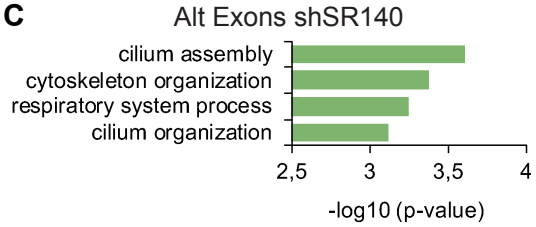

A
HeLa
U2OS
% of cells
Viable
Early apoptotic
Late apoptotic/necrotic
shCNT
shCNT #2
shSPF45 #1
shSPF45 #2
shSR140 #1
shSR140 #2
shCHERP #1
shCHERP #2
B
shCNT
shSPF45 #1
shSR140 #1
shCHERP #1
G1/S
Mitotic
G2
pH3S10
DNA content (PI)
pH3S10 (mitotic)

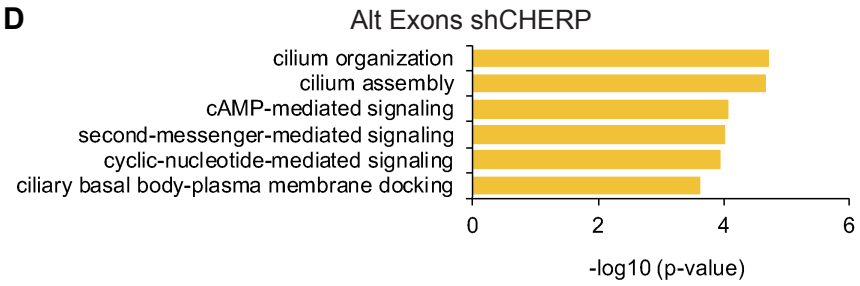

A
Differentially expressed genes
shSPF45
shSR140
shCHERP
788
537
732
556
831
718
Downregulated
Upregulated
B
Differentially expressed genes
shSPF45
shCHERP
shSR140
519
229
373
204
937
532
179
C
Gene ontolgy of differentially expressed genes
shSPF45
extracellular space
intrinsic component of plasma membrane
cytosolic large ribosomal subunit
proteinaceous extracellular matrix
extracellular matrix
integral component of plasma membrane
plasma membrane part
apical dendrite
extracellular region
anchoring junction
0
2
4
6
shSR140
extracellular space
extracellular region
plasma membrane part
plasma membrane
proteinaceous extracellular matrix
extracellular region part
intrinsic component of plasma membrane
integral component of plasma membrane
collagen trimer
intrinsic component of membrane
0
3
6
9
12
shCHERP
intrinsic component of plasma membrane
cell surface
integral component of plasma membrane
intrinsic component of membrane
plasma membrane part
integral component of membrane
extracellular space
membrane part
extracellular region
plasma membrane
0
3
6
9
12
15
-log10 (p-value)
D
shSPF45
GE
1035
209
AS
1617
shSR140
GE
1118
170
AS
1209
shCHERP
GE
1261
289
AS
1499

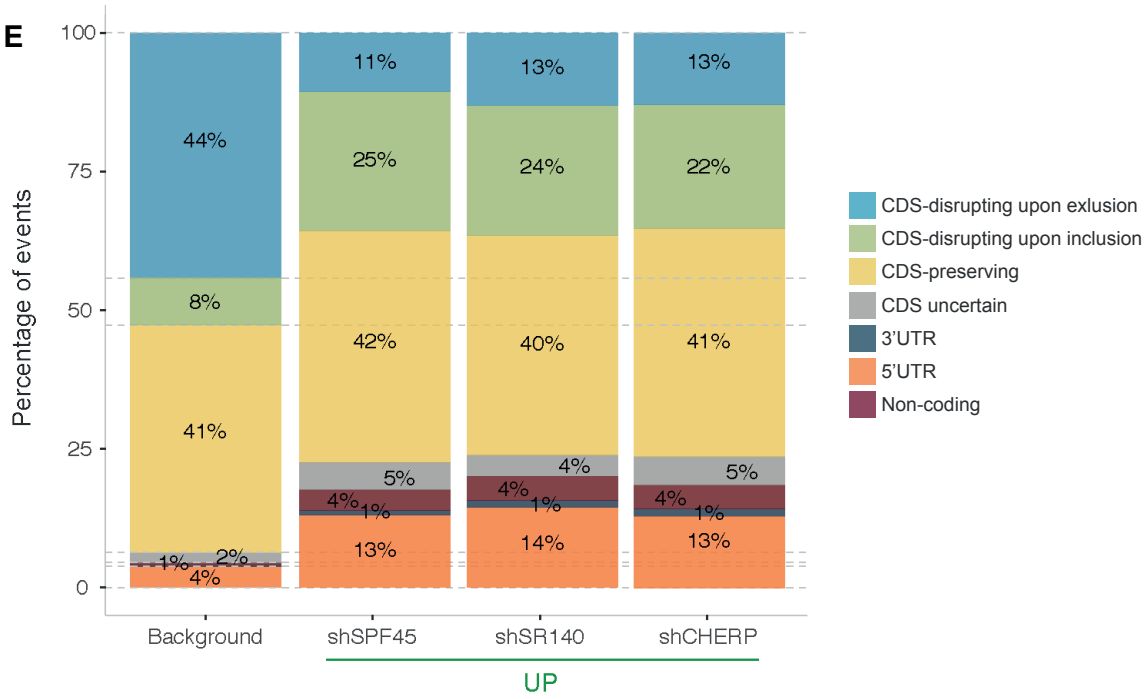

A
HeLa
ANAPC5 3'ss
FMNL1 E5
LMO7 3'ss
CLASP1 E24
INCENP E11
AURKB E2
CHEK1 E3
MST1R I5
PSI
shCNT
shSPF45
shSR140
shCHERP
sh #1
sh #2
B
U2OS
FMNL1 E5
LMO7 3'ss
CLASP1 E24
INCENP E11
AURKB E2
CHEK1 E3
C
All events
shSPF45
shSR140
shCHERP
1364
534
473
549
557
184
1307
Alt 3'ss
67
19
29
47
31
17
62
Alt 5'ss
34
7
11
5
23
3
38
RI
749
206
208
166
253
61
438
D
shSPF45
shSR140
shCHERP
Number of CEx events
ΔPSI ≥ 15%
ΔPSI ≤ -15%
827
827
656
656
551
551
407
339
407
279
328
279
146
286
146
144
286
144
545
545
251
251
950
950
E
Exon length
DOWN
UP
Triple overlap
non-changing
Triple overlap
Exon Length
Average ΔPSI
F
Intron length
UP
DOWN
shSPF45
shSR140
shCHERP
Triple overlap
Longer
Shorter
(relative to non-changing alternative exons)

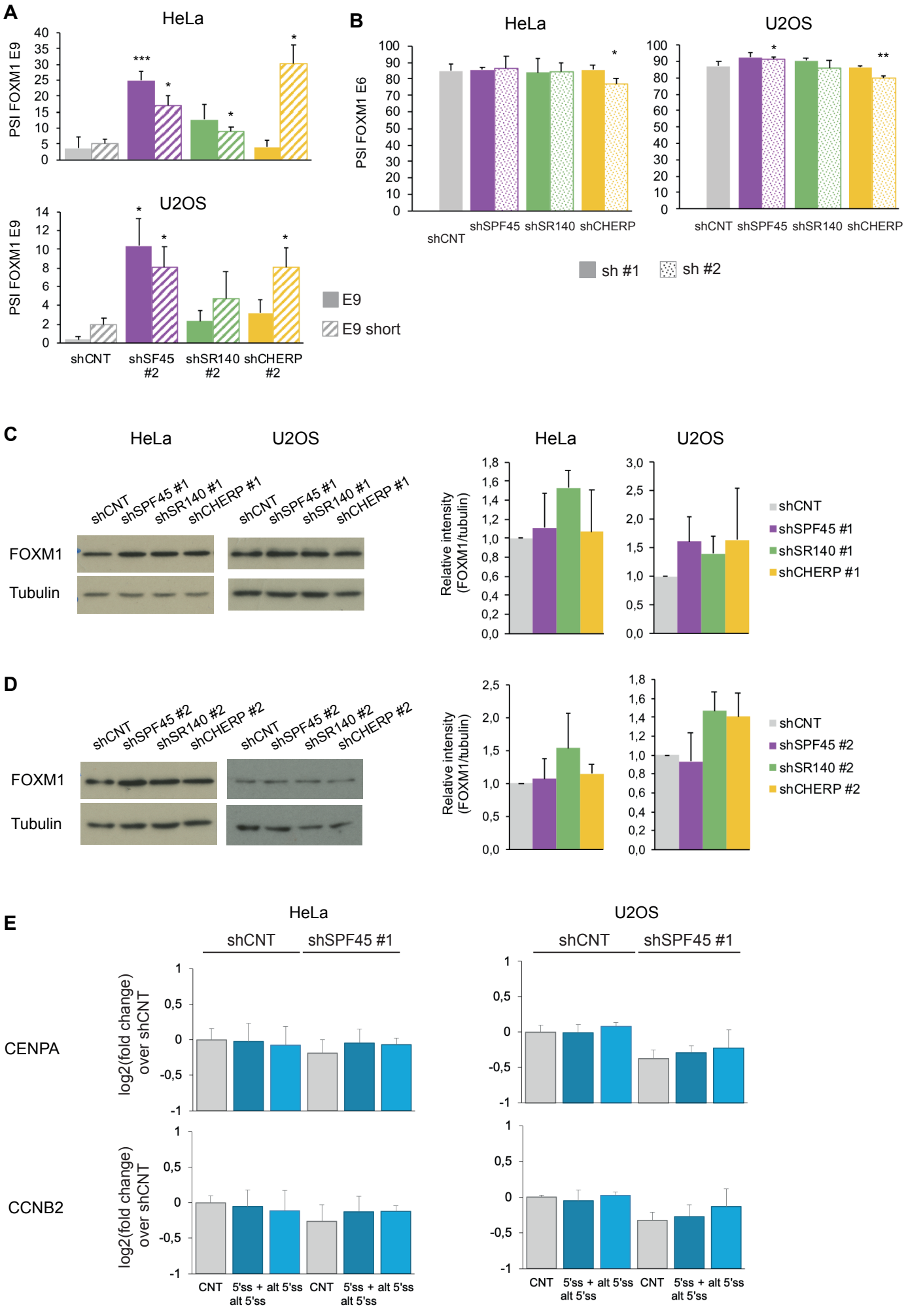

A
Sequence features
GC content
Length
5'ss
BP
Py strength
Py length
3'ss
RETAINED
SPLICED
shSPF45
shSR140
shCHERP
Triple overlap
(relative to non-changing introns)
Weaker/ shorter
Stronger/ longer
Lower
Higher
Shorter
Longer
0 500 1000 1500


B
Intron length
SPLICED
RETAINED
Triple overlap
non-changing
Triple overlap
Intron Length
Average ΔPSI


C
Alt Exons shSR140
cilium assembly
cytoskeleton organization
respiratory system process
cilium organization
-log10 (p-value)


D
Alt Exons shCHERP
cilium organization
cilium assembly
cAMP-mediated signaling
second-messenger-mediated signaling
cyclic-nucleotide-mediated signaling
ciliary basal body-plasma membrane docking
-log10 (p-value)


E
Percentage of events
Background
shSPF45
shSR140
shCHERP
UP
CDS-disrupting upon exlusion
CDS-disrupting upon inclusion
CDS-preserving
CDS uncertain
3'UTR
5'UTR
Non-coding


A
HeLa
PSI FOXM1 E9
U2OS
shCNT
shSF45 #2
shSR140 #2
shCHERP #2
E9
E9 short
B
HeLa
U2OS
PSI FOXM1 E6
shCNT
shSPF45
shSR140
shCHERP
sh #1
sh #2
C
HeLa
U2OS
shCNT
shSPF45 #1
shSR140 #1
shCHERP #1
FOXM1
Tubulin
Relative intensity (FOXM1/tubulin)
D
shSPF45 #2
shSR140 #2
shCHERP #2
E
CENPA
CCNB2
log2(fold change) over shCNT
CNT
5'ss + alt 5'ss
alt 5'ss