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Supplemental Information

Functional Network Analysis Reveals the Relevance of SKIIP in the Regulation of Alternative Splicing by p38 SAPK

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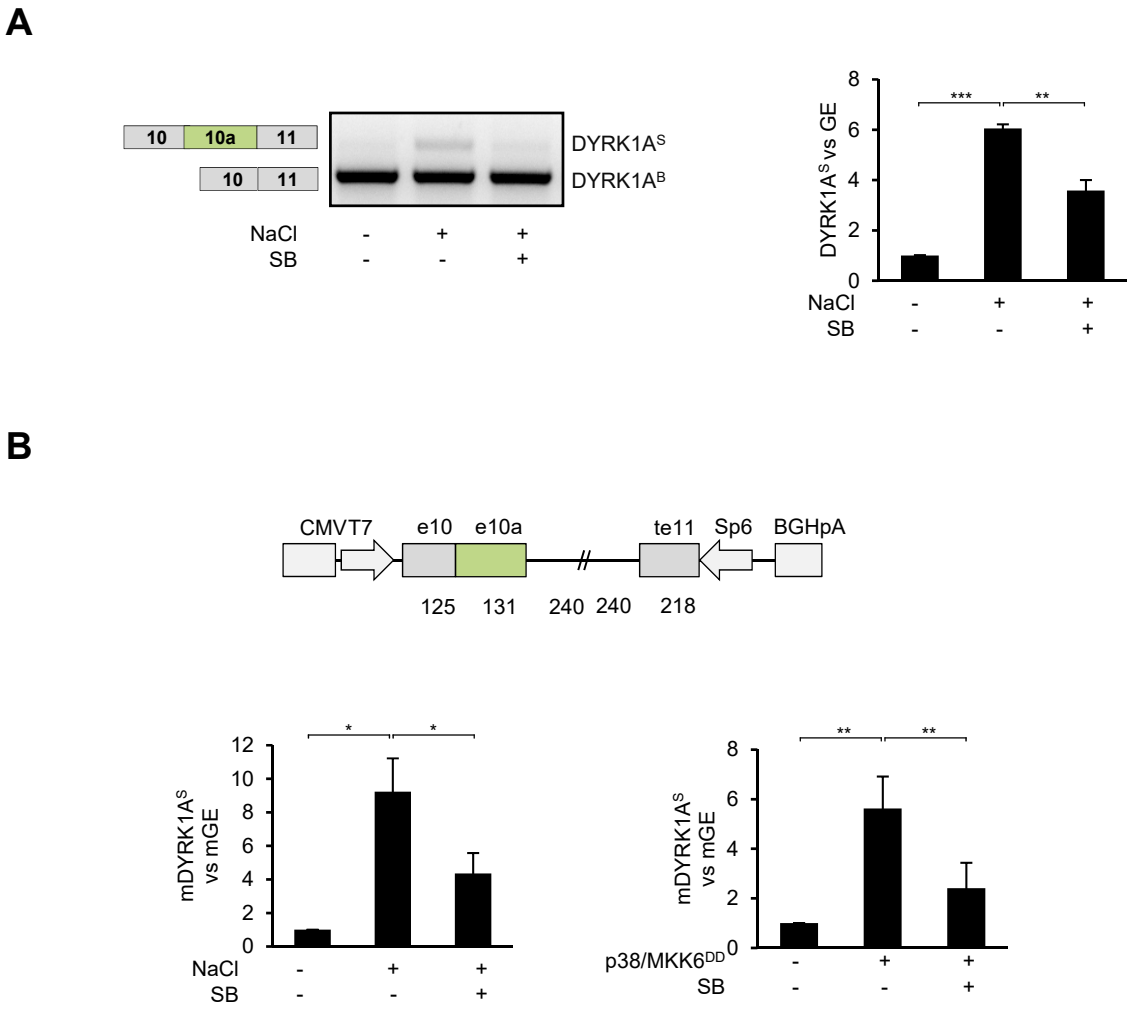

Figure S1. p38 SAPK modulates DYRK1A endogenous and minigene AS. Related to Table S1 and STAR methods. **(A)** Validation of AS event in DYRK1A (11.6.2) depicted with a schematic diagram, showing constitutive exons 10 and 11 (grey boxes) and alternative exon 10a (green box). HeLa cells were untreated, treated for 2 hours with 100 mM NaCl or pre-treated for 30 minutes with SB203580 prior to NaCl treatment. Changes in DYRK1A AS were assessed by RT-PCR using primers flanking constitutive exons (left panel). Levels of stress-induced isoforms (S) and total gene expression (GE) were determined by qPCR using primers targeting exon-junctions and constitutive exons, respectively (right panel). **(B)** Schematic diagram of the DYRK1A minigene used is shown. For analysis of the DYRK1A 5'ss event, the region from exon 10 to 220 bp upstream of the 3'ss of exon 11 was cloned, including a 5-kb deletion of intron 10. The length (bp) of the exons and introns included in the minigene is indicated under the diagram. Left panel: HeLa cells transiently transfected with DYRK1A minigene construct were treated as in (A). Right panel: HeLa cells were transiently transfected with DYRK1A minigene, p38 and $MKK6^{DD}$ and incubated (or not) with SB203580 for 24 hours. Minigene (m) AS isoforms and total minigene expression (mGE) were assessed by qPCR and normalized to non-treated cells, which were assigned a value of 1. Data are shown as means ±SD from at least three biological replicates. Significant changes were calculated by Student's t-test.

Figure S2

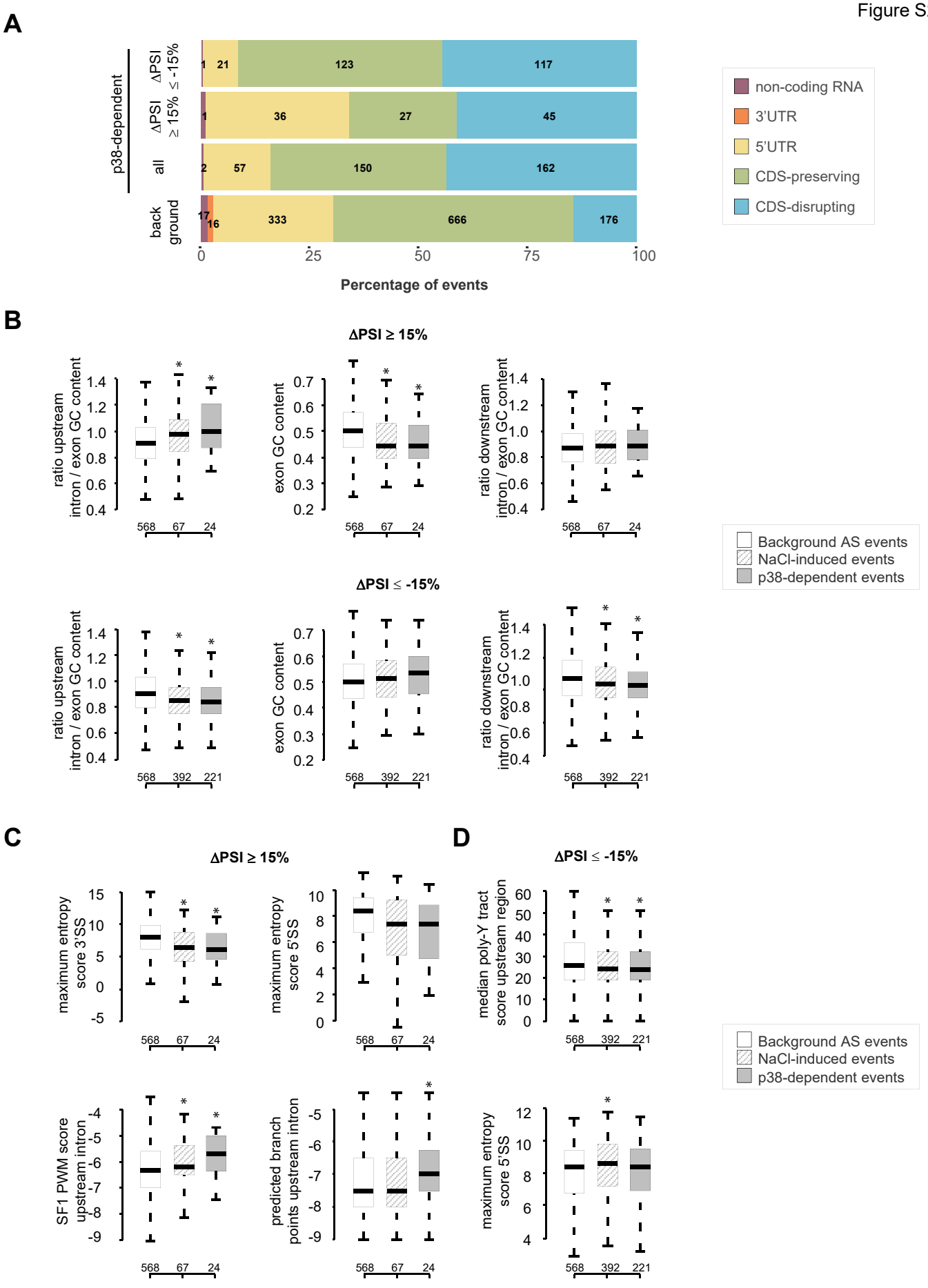
A
p38-dependent
ΔPSI ≤ -15%
ΔPSI ≥ 15%
all
back ground
1 21 123 117
1 36 27 45
2 57 150 162
17 16 333 666 176
0 25 50 75 100
Percentage of events
non-coding RNA
3'UTR
5'UTR
CDS-preserving
CDS-disrupting
B
ΔPSI ≥ 15%
ratio upstream intron / exon GC content
exon GC content
ratio downstream intron / exon GC content
568 67 24
ΔPSI ≤ -15%
568 392 221
Background AS events
NaCl-induced events
p38-dependent events
C
ΔPSI ≥ 15%
maximum entropy score 3'SS
maximum entropy score 5'SS
SF1 PWM score upstream intron
predicted branch points upstream intron
D
ΔPSI ≤ -15%
median poly-Y tract score upstream region
maximum entropy score 5'SS

Figure S2. NaCl-induced and p38-dependent exons feature distinct intronic and exonic GC content and weaker splice sites. Related to Figure 1 and Table S2. **(A)** Proportion of events disrupting the transcript open reading frame (CDS-disrupting), preserving it (CDS-preserving), mapping in 3'/5' untranslated regions (UTR) or in non-coding RNA. Numbers of events are shown for each category: background alternative events, p38-dependent events (ALL) and subsets of p38-dependent events with ΔPSI ≥ 15 or ΔPSI ≤ -15. **(B)** Exonic GC content and ratio intronic / exonic GC content in NaCl-induced and p38-dependent exons compared to background. Top and bottom panels represent exons included and skipped upon NaCl treatment, respectively. **(C-D)** Splice site strength and branch point features in NaCl-induced and p38-dependent included (C) or skipped (D) exons after osmostress compared to background alternative exons. Boxplots corresponding to each feature represent the inter-quantile range (IQR) with median values shown by the black line. Outliers were discarded. Significant changes were calculated by Mann-Whitney-Wilcoxon test comparing each set of exons vs. the background set (*$p<0.05$).

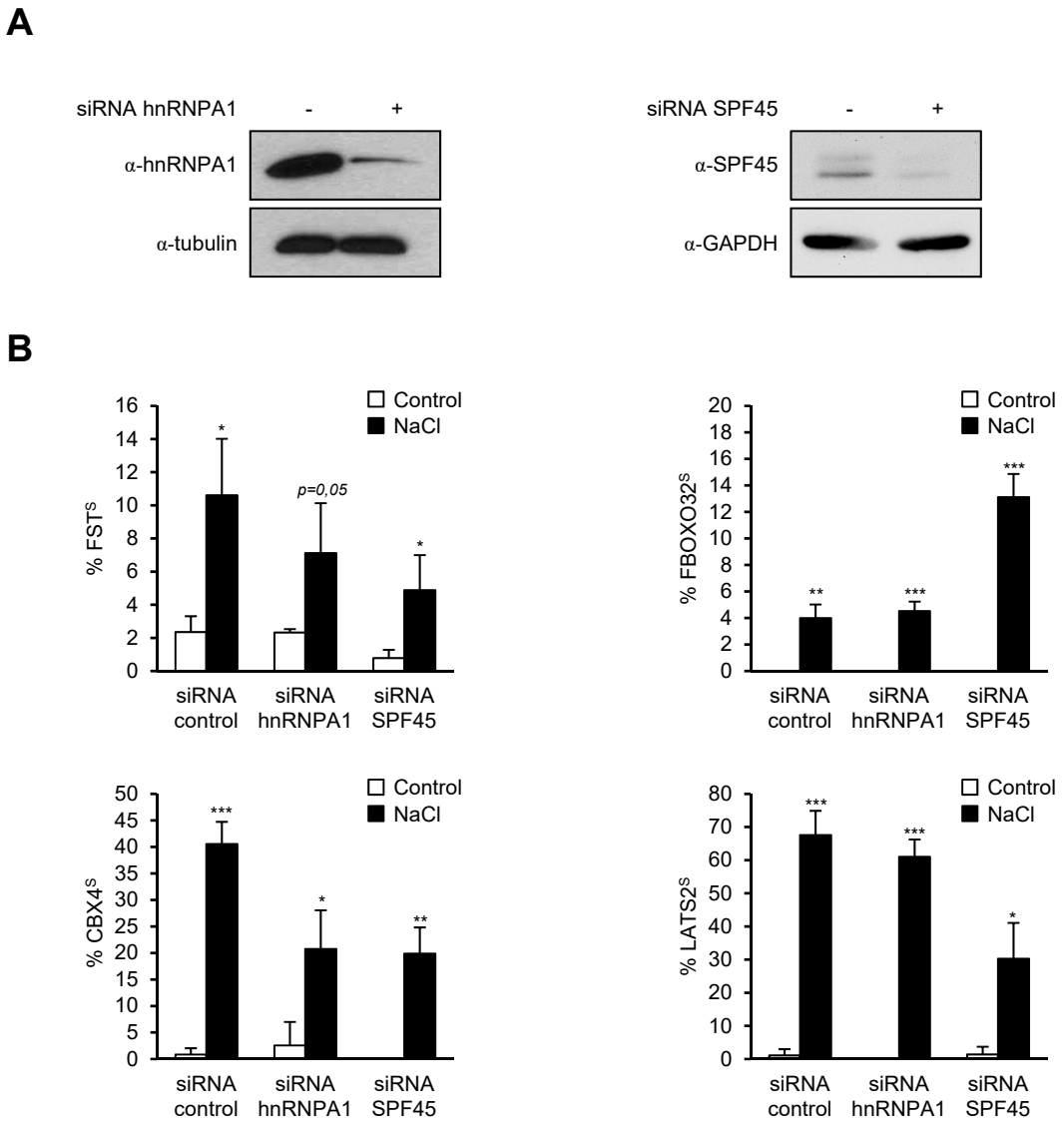

Figure S3. Depletion of hnRNPA1 or SPF45 does not prevent significant changes in p38-dependent events upon stress. Related to Figure 3. **(A)** Western blot analysis of HeLa cells expressing control, hnRNPA1 or SPF45 siRNAs. **(B)** siRNA-treated HeLa cells were stimulated or not for 3 hours with 100 mM NaCl. AS patterns were analyzed by RT-PCR as in Figure 1.

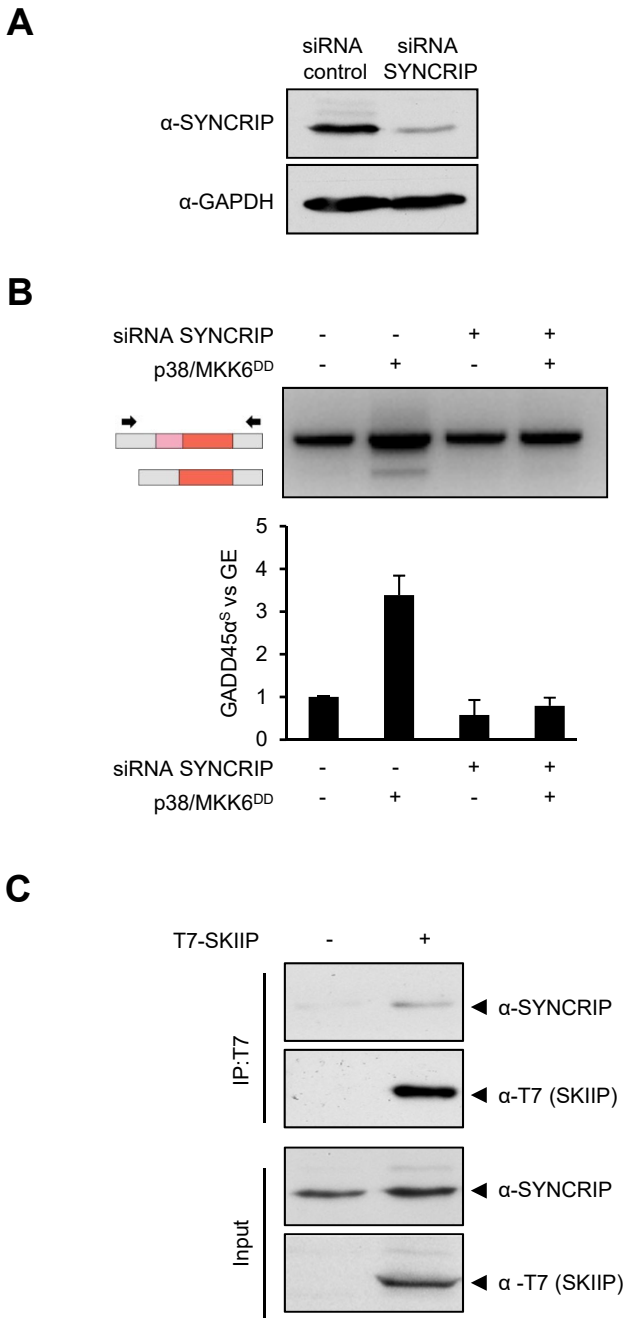

Figure S4. SYNCRIP is essential for p38-dependent GADD45α^S induction. Related to Figure 3. **(A)** Western blot analysis of HeLa cells expressing control or SYNCRIP siRNA is shown. **(B)** SYNCRIP is essential for p38-dependent GADD45α^S induction. HeLa cells were treated with scrambled or SYNCRIP stealth siRNA and treated for 2 hours with 100 mM NaCl. Changes in GADD45α AS were assessed by RT-PCR using the primers indicated by arrows (upper panel). Levels of stress-induced isoforms (S) and total gene expression (GE) of GADD45α were determined by qPCR using primers targeting exon-junctions and constitutive exons, respectively (lower panel). Relative expression of stress isoforms was quantified as fold change over non-treated cells. **(C)** SYNCRIP interacts with SKIIP *in vivo*. T7-SYNCRIP was expressed in HeLa cells, immunoprecipitated with anti-T7 coupled Sepharose beads and analyzed by Western blotting with anti-T7 and anti-SYNCRIP antibodies.

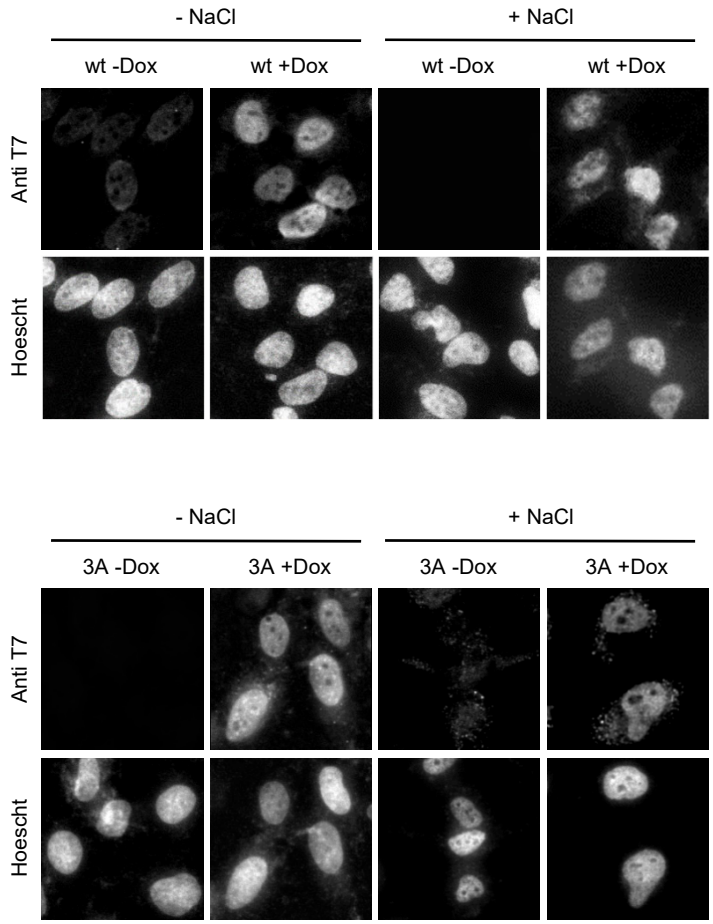

Figure S5. SKIIP nuclear localization is not affected in response to osmostress or by its phosphorylation by p38. Related to Figure 5. HeLa cells stably expressing doxycycline-inducible T7-SKIIP and T7-SKIIP 3A proteins were grown in 8-chamber glass slides. The expression of T7-epitope tagged SKIIP and SKIIP 3A was induced (+Dox) or not (-Dox) upon the addition of doxyciclin (12.5 µg/ml for 24 hours) and cells were stressed (100 mM NaCl for 1 hour). Images of the immunocytochemistry with anti T7 antibody in the presence of Hoechst 33342 to stain the nuclei are shown.

EventID	Gene name	Type of event
00312.0003.1	BUB1B	novel_exons
00058.0052.1	CD44	exon(s)skipped
00058.0007.4	CD44	exon(s)skipped
00246.0016.1	SPTAN1	alt_splice_acceptor
00351.0008.1	MAP2K1	exon(s)skipped
274:1_5:1	FN1	novel_exons
165:1_5:33	CROP	Mutually-Exclusive-Exons
11.6.2	DYRK1A	novel_exons
116:1_4:1	CDC40	novel_exons
00111.0016.1	FOS	exon(s)skipped

Table S1. List of AS events osmostress- and SB203580- dependent (custom splicing-sensitive microarray platform). Related to STAR methods.