The centrosomal kinase NEK2 is a novel splicing factor kinase involved in cell survival

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Supplementary Table S1 and Supplementary Figures S1-S6

Supplementary Table S1: Sequence of si-RNAs and primers used in this study

siRNAs	
Oligo name	**Sequence (5'→3')**
si-SCR	UCUUUCUUCUGCUUUGCGG
si-NEK2	GCAGACAGAUCCUGGGCAU'
	GGAUCUGGCUAGUGUAAUU'
	GGACCUACUUAGAUGAAGA
	GCUAGAAUAUUAAACCAUG
si-SRSF1	CCAAGGACAUUGAGGACGU
Primers	
Oligo name	**Sequence (5'→3')**
NEK2 FW H1077	GGAACGGAAGTTCCTGTC
NEK2A/C REV H1229R	CACTTGGACTTAGATGTGA
NEK2B REV AF008	GGCGAATTCCATACCGTTAC
HPRT FW	TGACCAGTCAACAGGGGACA
HPRT REV	TTCGTGGGGTCCTTTTCACC
E1A-569 FW	ATTATCTGCCACGGAGGTGT
E1A-1315 REV	GGATAGCAGGCGCCATTTTA
BCL-X Exon2 FW (BCL-X minigene)	CCATGGCAGCAGTAAAGCAA
BGH REV (BCL-X minigene)	CAGTGGGAGTGGCACCTTC
GAPDH FW	CCCTTCATTGACCTCAACTACATG
GAPDH REV	TGGGATTTCCATTGATGACAAGC
BCL-X SHORT FW	GGGACAGCATATCAGAGCTTTGAACAGGATAC
BCL-X LONG FW	AGGAGAACGGCGGCTGGGATAC
BCL-X REV	TCATTTCCGACTGAAGAGTGAGCCCA
hBIN1 ex12 FW	GGAAAGGCCCACCAGTCC
hBIN1 ex 12 REV	AACGTGTCCTCAAACAGGCT
hBIN1 ex 14 FW	CTCTCTTCCTGCTGTCGTGG
hBIN1 ex 14 REV	GGGGGCAGGTCCAAGCG
MNK2 ex 12 FW	GCGCTGCCAAAGACCTCATCTCC
MNK2a REV	ACGTGAGGTCTTTGGCACAGCT
MNK2b REV	GGAAGTGACTGTCCCACCTCTGC

Supplementary Figure Legends

Supplementary Figure S1. NEK2 nuclear localization in cancer cells. (A) Immunofluorescence analysis of TCam-2 (seminoma), PC-3 (prostate), Caco-2 (colon) and HeLa (cervix) cancer cells stained with rabbit anti-NEK2 (red) antibody and Hoechst (blue) and analysed by fluorescence microscope. Scale bar = 10 μm.

Supplementary Figure S2. NEK2 spliced-variants expression and localization. (A). Schematic representation of the *NEK2* gene and of its alternative splice variants, NEK2A, NEK2B, and NEK2C. Alternative polyadenylation signal, alternative 3' splice sites (SS) and residue number are indicated. Black arrows indicate primers used for the RT-PCR analysis. All three NEK2 variants are identical in their N-terminal kinase domain and leucine zipper (LZ) dimerization motif. NEK2B is the shortest isoform, bearing some unique residues (371-384) encoded by part of intron 7 and lacking the coiled coil (CC) domain and the APC degradation motifs present in NEK2A and C. NEK2C shows a short deletion (Δ371-378) in the C-terminus, creating a strong nuclear localization signal (NLS) (15). (B) RT-PCR amplification of *NEK2* spliced-variants from HeLa (cervix), MCF7 (breast), PC-3 (prostate), TCam-2 (seminoma) cancer cells (upper panel). PCR products were separated on 10% polyacrylamide gel and stained with ethidium bromide. *HPRT* expression levels were evaluated to normalize for RNA content. Bar graph (lower panel) represents densitometric analysis for the NEK2A/C ratio (mean ± SD, n=3). (C) HeLa cells were transfected with expression vectors for the GFP-tagged NEK2 spliced-variants and fixed 24h after transfection for fluorescence-microscopy analysis. Nuclei were stained with Hoechst (blue). Scale bar = 20 μm.

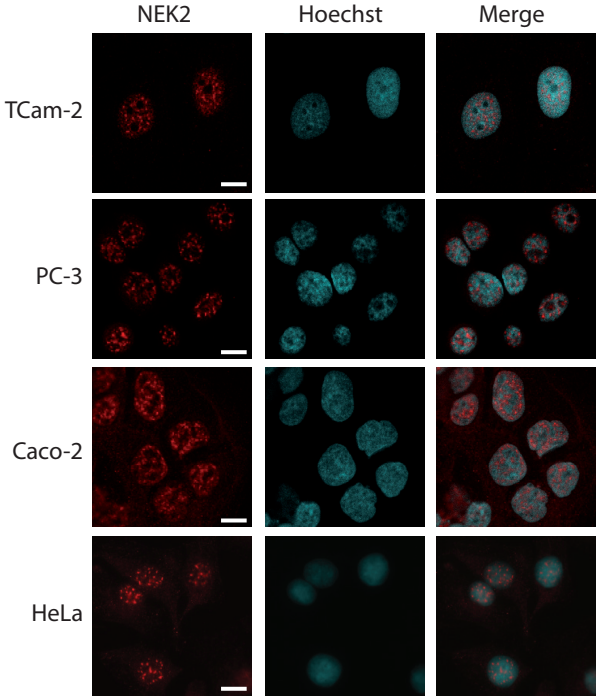
Supplementary Figure S3. NEK2A phosphorylates SRSF1 *in vivo*. (A,B) Western Blot analysis with anti-GFP and anti-FLAG antibodies of the immunoprecipitation assay performed with control mouse IgG or anti-FLAG antibody of nuclear extracts of HEK293T expressing FLAG-SRSF1 and GFP-NEK2A (A) or of total extracts of HEK293T expressing FLAG-SRSF1 and GFP-NEK2B (B).

(C) HEK 293T cells were transfected with GFP-SRSF1, wild-type NEK2A (WT) or kinase-dead NEK2A K37R (KD) vectors. Cell lysates were resolved on SDS-PAGE and expression of recombinant proteins was detected by Western Blot using anti-GFP antibody. ACTIN was used as loading control.

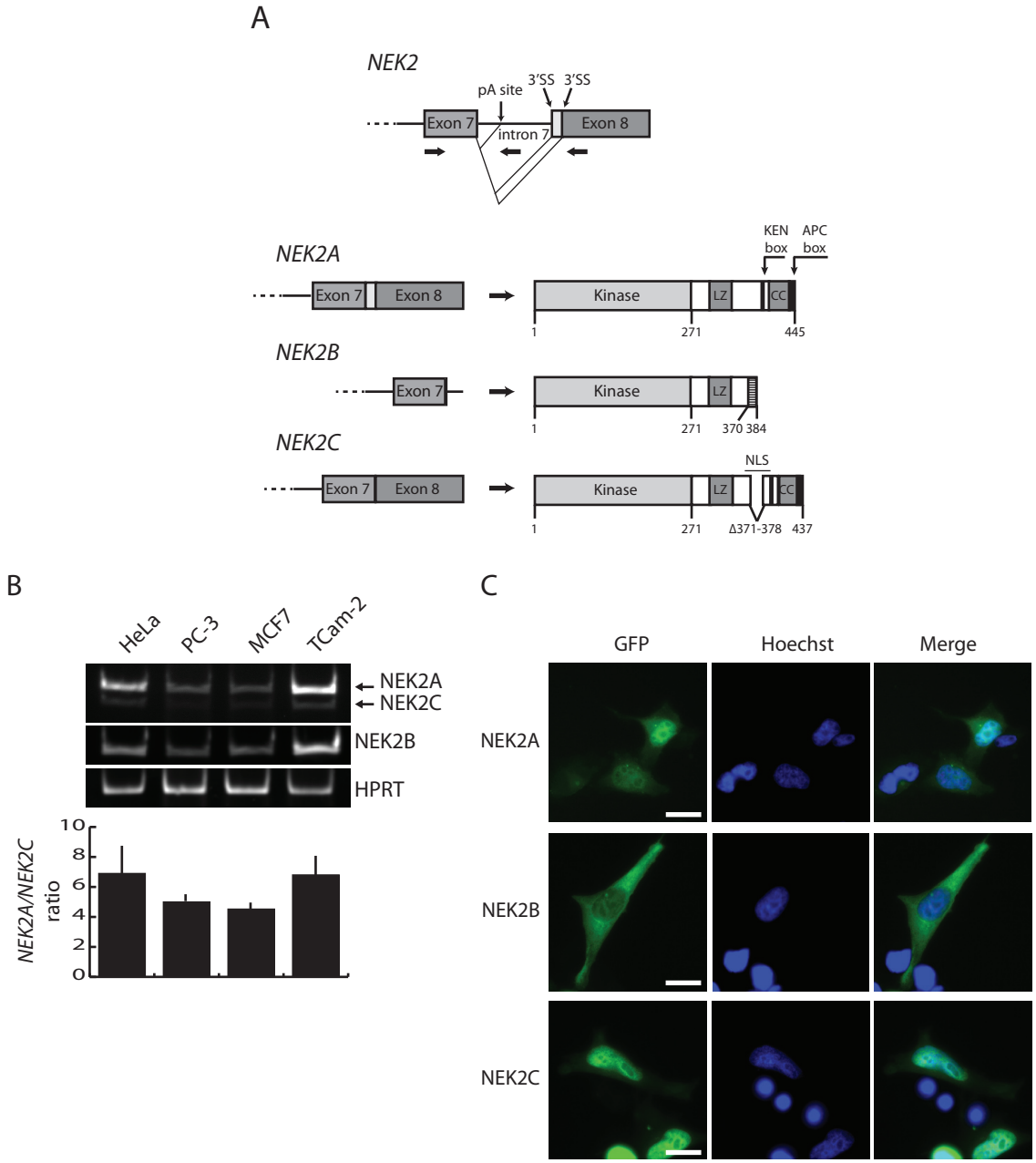
Supplementary Figure S4. NEK2 expression promotes cell survival. (A) qPCR analysis of the splicing pattern of *MKNK2* and *BIN 1* in HeLa cell transfected with empty vector, GFP-NEK2C or GFP-SRSF1. Bar graphs represent the indicated isoform ratio (mean ± SD, n=3, *p<0,05). (B,C) Western Blot analysis of PARP1 cleavage (B) and quantitative analysis of cleaved-CASPASE 3 immunofluorescence (bar graph represents mean ± SD, n=3, *P<0,05) (C) in HeLa cells transfected with either scramble (si-SCR) or NEK2 (si-NEK2) siRNAs and serum-starved for 8h. Western Blot analysis assessing NEK2 silencing efficiency was performed (B).

Supplementary Figure S5. NEK2C overexpression does not affect SRSF1 subcellular localization. Immunofluorescence analysis of HeLa (A) and HEK 293T (B) cells transfected with the GFP-SRSF1 vector in presence or not of the pCDNA3N2myc-NEK2C WT vector. Nuclei were stained with Hoechst (blue). Scale bar = 20 μm.

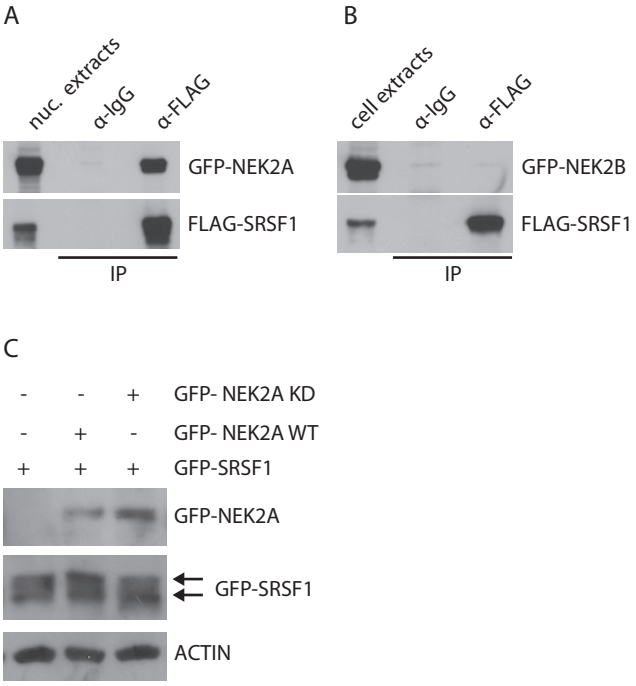
Supplementary Figure S6. Modulating NEK2 expression levels does not affect AKT activation. (A,B) Western Blot analysis for AKT phosphorylation status, using anti-AKT and anti-pAKT Ser473 antibodies. (A) HeLa cells were transfected with FLAG-SRPK1 or GFP-NEK2C WT vectors and harvested 24h after transfection. (B) HeLa cells were transfected with scramble (si-SCR), *NEK2* (si-NEK2) or *SRPK1* (si-SRPK1) siRNAs and harvested 48h after transfection.



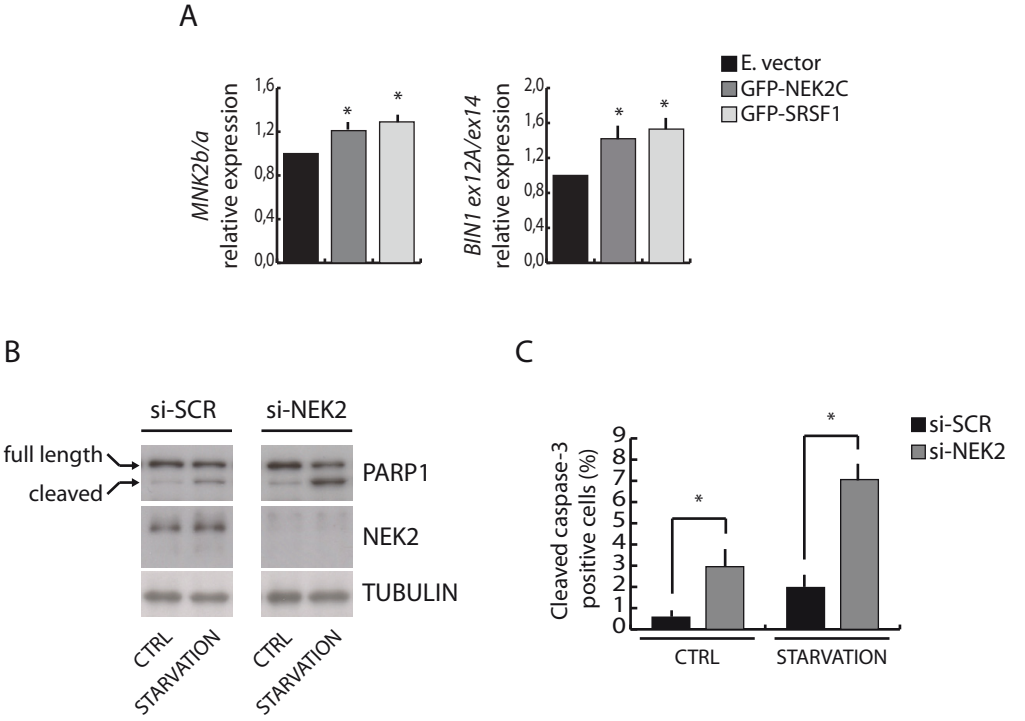
Supplementary Figure 1



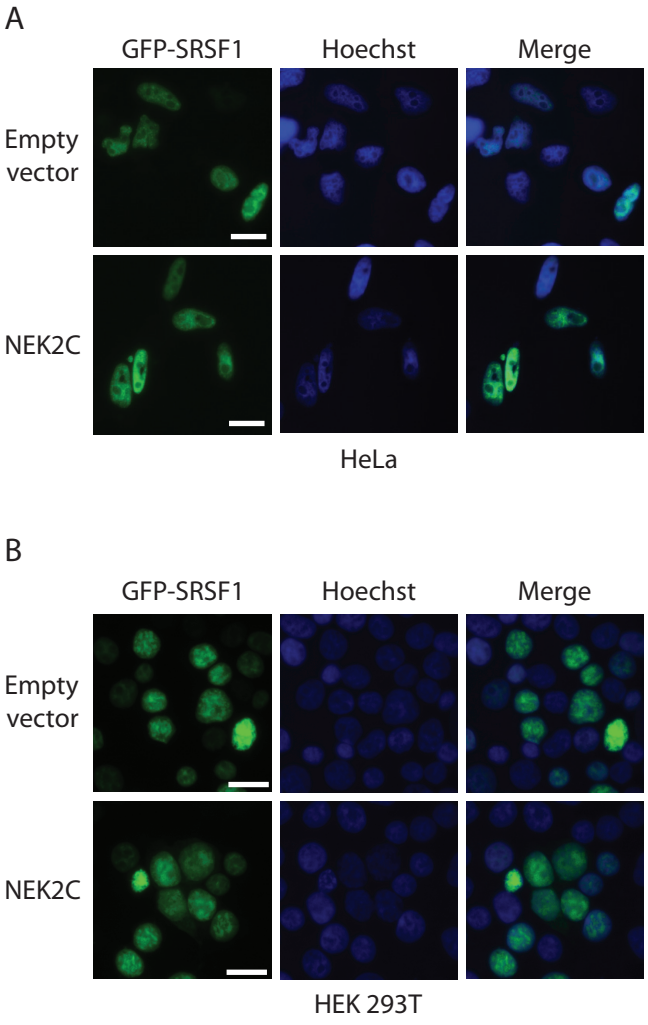
Supplementary Figure 2



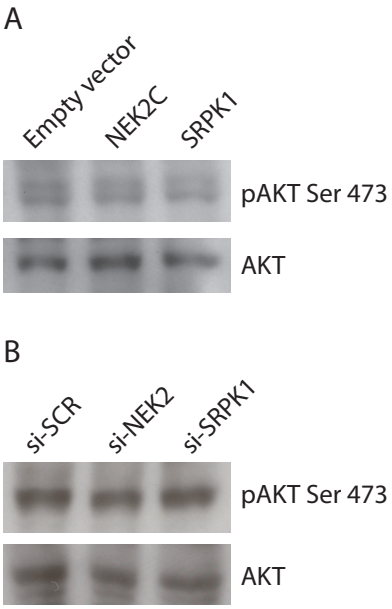
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6