Expanded View Figures

Figure EV1. Lack of centrioles affect DNA repair by homologous recombination.

A, B U2OS cells were treated with 125 nM of centrinone, or DMSO as a control, for 24 h (A) or 7 days (B). Then, the presence of centrosomes was immunodetected using an antibody against the centriolar proteins γ-tubulin and CP110. The percentage of cells without centrioles was counted and plotted.

C U2OS cells treated for 7 days with centrinone or DMSO, as indicated, were exposed to Camptothecin 1 μM for 1 h and prepared for immunofluorescence using an anti-RPA antibody as described in the Materials and Methods section. The number of RPA foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative experiment out of three performed with similar results is shown.

D–F (D) RPE-1 cells treated for 7 days with centrinone or DMSO, as indicated, were irradiated with 10 Gy and 1 h later prepared for immunofluorescence using an anti-RPA antibody as described in the Materials and Methods section. The number of RPA foci per cell was calculated as in (C). The number of RPA foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative biological replicate out of three that rendered similar results is shown. (E) As in (D), but in U2OS cells treated for 24 h with centrinone or DMSO. (F) As in D, but in p53 KO or p53 KO HsSAS-6 KO RPE-1 cells.

G U2OS cells transfected with a plasmid harboring a mCherry-tagged PLK4, or an empty vector as a control, were grown for 3 days. Then, the number of centrosomes was immunodetected using an antibody against the centriolar proteins γ-tubulin and CP110. The number of cells with more than four centrioles was counted and plotted.

H Same as Fig 1A, but in cells overexpressing or not PLK4.

I Same as Fig 1B, but in cells overexpressing or not PLK4.

J U2OS cells treated for 7 days with centrinone or DMSO, as indicated, were prepared for immunofluorescence using an anti-γH2AX antibody as described in the Materials and Methods section. The number of γH2AX foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative experiment out of three performed with similar results is shown.

K DSB repair kinetic using γH2AX as a proxy. U2OS cells exposed to centrinone or DMSO as a control for 7 days were irradiated (2 Gy). Samples were collected at the indicated time points and γH2AX was immunodetected using a specific antibody as described in the Materials and Methods section. NI indicates a non-irradiated sample taken just before irradiation. The number of γH2AX foci was scored and plotted.

L Same as Fig 1C, but using an antibody against the NHEJ factor RIF1. The number of RIF1 foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative biological replicate out of three that rendered similar results is shown.

M Same as Fig 1C, but in cells treated with Taxol, Nocodazole or DMSO. The number of RPA foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative biological replica out of three that rendered similar results is shown.

N Cell cycle distribution of samples treated for 7 days with centrinone or DMSO, as indicated.

O, P (O) U2OS cells treated for 7 days with centrinone or DMSO, as indicated, were fixed and the percentage of mitotic cells was quantified based on DAPI staining. At least 400 cells were quantified per condition. (P) Same as (O) but cells were immunostained with an antibody against phospho-H3S10. At least 400 cells were scored per condition and the percentage of phospho-H3S10 positive cells was quantified.

Q Cell viability of U2OS cells treated for 7 days with centrinone or DMSO was quantified by Trypan Blue staining and plotted.

R–T Same as Fig 1C–E but using Saos-2 cells.

U–W Same as Fig 1C–E but using RPE-1 p53 KO cells.

X–Z Same as Fig 1C–E but using RPE-1 p21 KO cells.

Data information: (A, B, G–I, K, O–Q) The average and standard deviation of three independent experiments is shown. The statistical significance was calculated using a Student's *t*-test. (C–F, J, L, M, R–Z) One representative experiment out of three performed with similar results is shown. Error bars represent standard deviations. *P*-values are represented with two ($P < 0.01$), three ($P < 0.001$) or four ($P < 0.0001$) asterisks. Non-statistical significance is labeled ns. (R–Z) The number of RPA foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative biological replicate out of three that rendered similar results is shown.

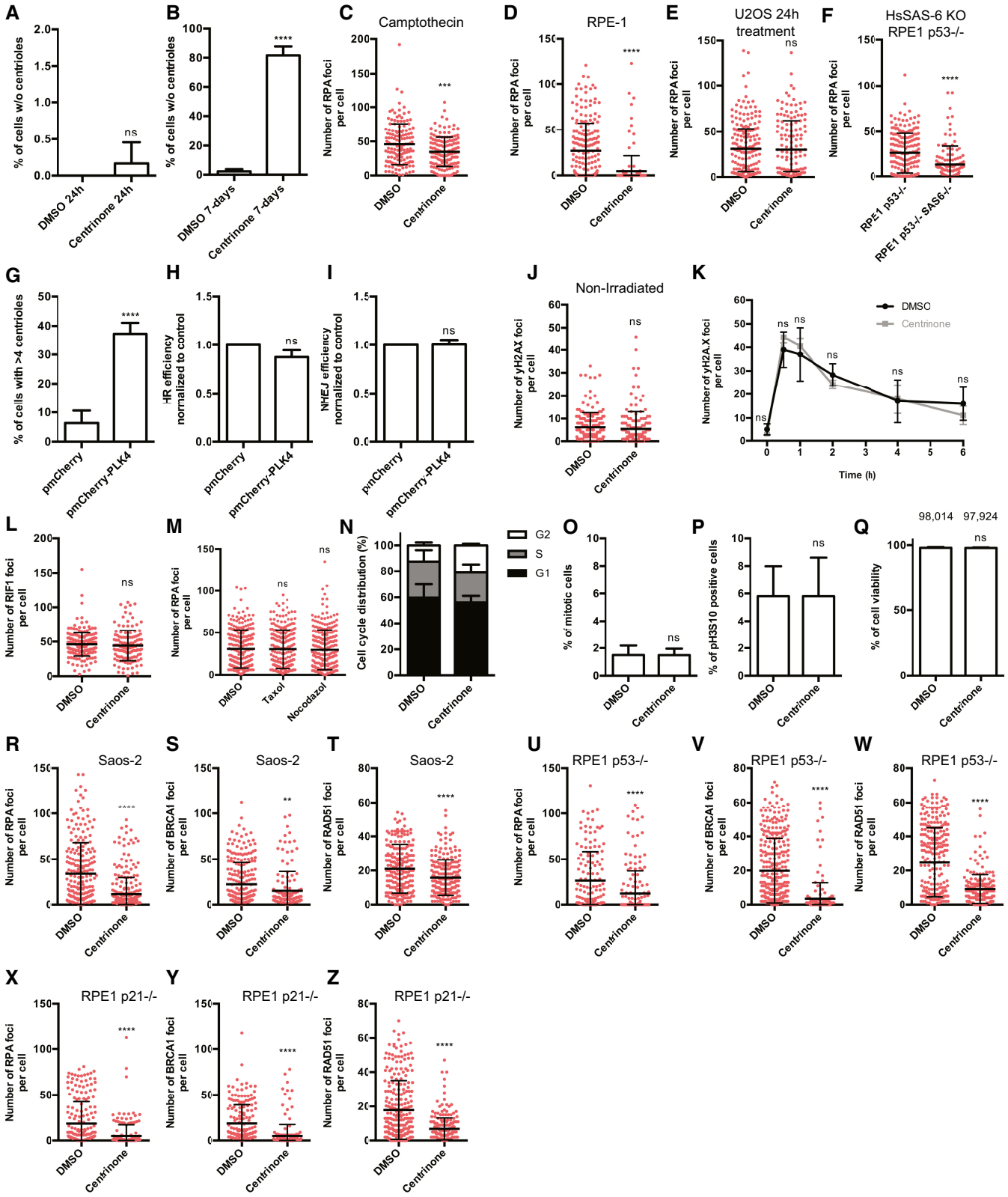


Figure EV1.

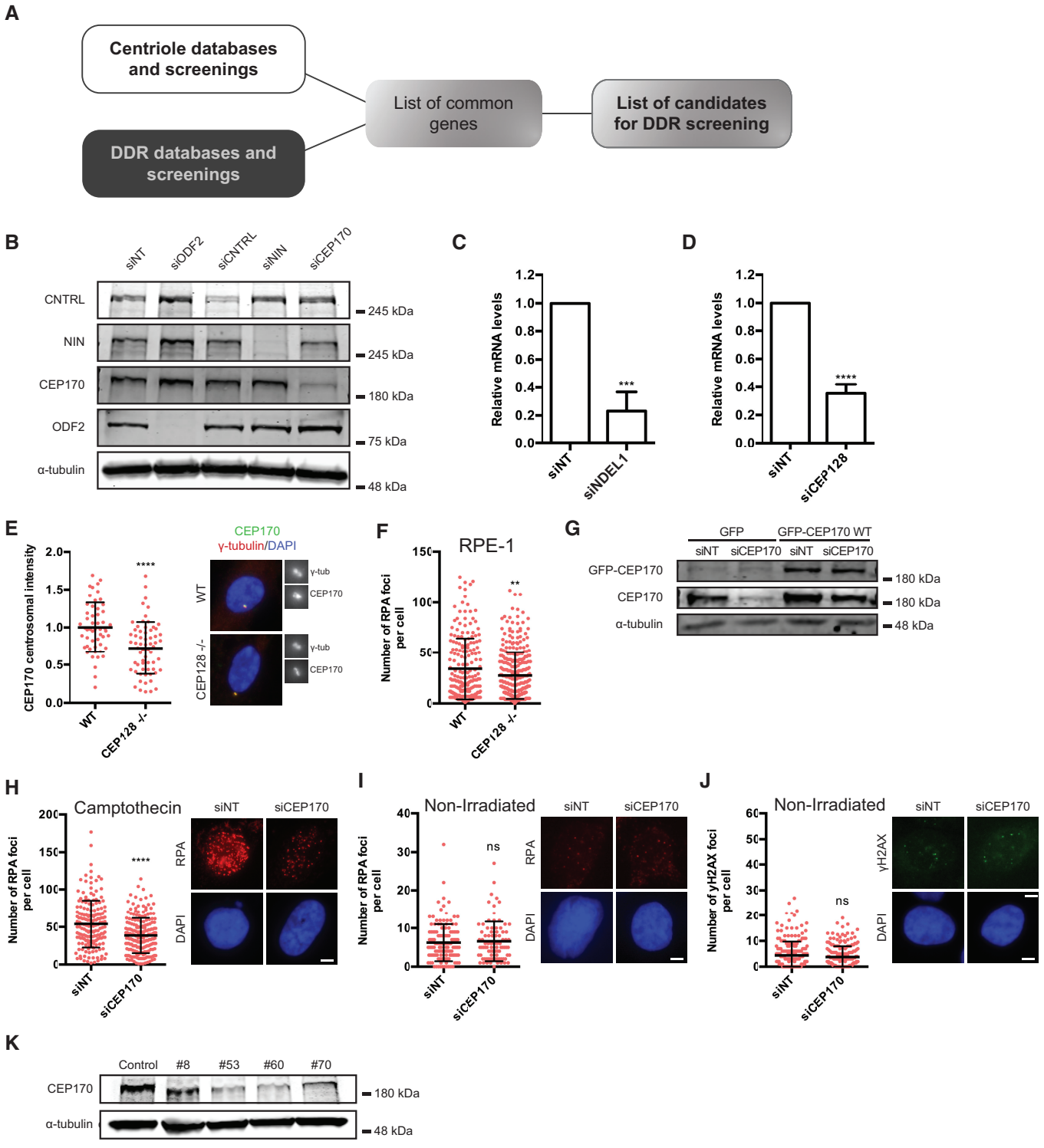

A
Centriole databases and screenings
DDR databases and screenings
List of common genes
List of candidates for DDR screening
B
siNT
siODF2
siCNTRL
siNIN
siCEP170
CNTRL
NIN
CEP170
ODF2
α-tubulin
245 kDa
245 kDa
180 kDa
75 kDa
48 kDa
C
Relative mRNA levels
siNT
siNDEL1
D
Relative mRNA levels
siNT
siCEP128
E
CEP170 centrosomal intensity
WT
CEP128 -/-
CEP170
γ-tubulin/DAPI
γ-tub
CEP170
F
RPE-1
Number of RPA foci per cell
G
GFP
GFP-CEP170 WT
siNT
siCEP170
GFP-CEP170
CEP170
α-tubulin
180 kDa
180 kDa
48 kDa
H
Camptothecin
Number of RPA foci per cell
RPA
DAPI
I
Non-Irradiated
Number of RPA foci per cell
ns
J
Non-Irradiated
Number of γH2AX foci per cell
γH2AX
K
Control
#8
#53
#60
#70
CEP170
α-tubulin
180 kDa
48 kDa

Figure EV2.

Figure EV2. Identification of the subdistal appendages as involved in HR regulation.

A Pipeline for identification of candidate genes involved in the connection between the centrosome and the DDR.

B U2OS cells were transfected with the indicated siRNAs and 48 h later, protein samples were prepared, resolved in SDS-PAGE and blotted with the indicated antibodies.

C, D (C) U2OS cells were transfected with the indicated siRNAs. Forty-eight hours later, total RNA was isolated and the levels of the NDEL1 RNA were calculated using Q-PCR. The levels were normalized to the sample transfected with a control siRNA, taken as one. The average and standard deviation of three experiments is plotted. Statistical significance was calculated using a Student's *t*-test. (D) Same as (C) but in cells transfected with a siRNA against CEP128 and using Q-PCR primers against the mRNA for this protein.

E Same as Fig 2D but for RPE-1 CEP128 KO or RPE-1 control cells. The centriolar intensity of CEP170 was measured at least in 50 cells per condition and quantified using FIJI software and plotted. The average and standard deviation of one representative experiment out of three biological replicas that rendered similar results in shown.

F Same as Fig 1C but for RPE-1 CEP128 KO or RPE-1 control cells. The number of RPA foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative experiment out of three biological replicas that rendered similar results in shown.

G Protein samples were prepared from cells treated as in Fig 2F, resolved in SDS-PAGE and blotted with anti-GFP, anti-CEP170 and anti-alpha-Tubulin antibodies as indicated. Note that the faint band observed with the anti-GFP antibody in the first two lanes is unspecific. Also note that CEP170 and GFP-CEP170 bands could not be resolved with the anti-CEP170 antibody due to the high size of these proteins.

H Same as Fig EV1C but in CEP170 siRNA-depleted or siRNA control cells.

I, J Same as in Fig EV1J but for CEP170 siRNA-depleted or siRNA control cells stained with anti-RPA antibodies (I) or γH2AX antibodies (J).

K Protein samples were prepared from U2OS CEP170 heterozygous KO clones, resolved in SDS-PAGE and blotted with the indicated antibodies.

Data information: (C and D) The average and standard deviation of three independent experiments is shown. The statistical significance was calculated using a Student's *t*-test. *P*-values are represented with two ($P < 0.01$), three ($P < 0.001$) or four ($P < 0.0001$) asterisks. Non-statistical significance is labeled ns.

Figure EV3. CEP170 in response to DNA damage.

A A representative U2OS cell of a total of 20 inspected expressing GFP-CEP170 and exposed to laser micro irradiation (yellow dashed line). Cells were monitored over 1 h taking images every 5 min. The orange arrow points to centrosomal CEP170 signal. Scale bar 7 μm.

B U2OS cells stably expressing a GFP-CEP170 construct were irradiated (10 Gy, plus sign) or not (minus sign). Protein samples were prepared 1 h later by nuclear fractionation as described in the Materials and Methods section. Samples were resolved in SDS-PAGE and blotted with the indicated antibodies. Size ladder marker is shown on the right side. A representative image of three independent experiments is shown.

C, D Same as Fig 1D and E but for RPE-1 CEP128 KO or RPE-1 control cells.

E Total levels of endogenous CEP170 were immunodetected in protein samples from U2OS cells irradiated (10 Gy, +IR) or not (−IR) resolved in SDS-PAGE using an antibody against this protein. Size ladder marker is shown on the right side. A representative image of four independent experiments is shown on the top, and the average and standard deviation of the quantification of the western blots at the bottom.

F Alignment of human CEP170 sequence flanking Serine-637 (red box) with the homologous sequences from the indicated vertebrates.

G Protein samples were prepared from cells treated as in Fig 3H, resolved in SDS-PAGE and blotted with anti-GFP, anti-CEP170 and anti-Tubulin antibodies as indicated. Note that the faint band observed with the anti-GFP antibody in the first two lanes is unspecific. Also note that CEP170 and GFP-CEP170 bands could not be resolved with the anti-CEP170 antibody due to the high size of these proteins.

H Same as in Fig 3G but examples of ectopic centrosomal localization of GFP tagged CEP170 WT and mutant versions. Orange arrows point to subdistal appendage localization, white arrows to centriole proximal localization and blue arrows point to ectopic centrosomal localization of CEP170 variants. Scale bar 500 nm.

I Same as Fig 3H, but in cells pretreated for 1 h with inhibitors against ATM (ATMi), ATR (ATRi) or DMSO as a control.

J Same as Fig 1C but in cells treated with centrinone or DMSO and stably expressing the indicated GFP constructs.

Data information: (C, D, E, I, and J), The number of the indicated foci per cell for at least 200 cells per condition was quantified automatically using FIJI software and plotted. One representative experiment out of three biological replicates that rendered similar results is shown. Error bars represent standard deviation. The statistical significance was calculated using a Student's *t*-test. *P*-values are represented with two ($P < 0.01$), three ($P < 0.001$) or four ($P < 0.0001$) asterisks. Non-statistical significance is labeled ns.

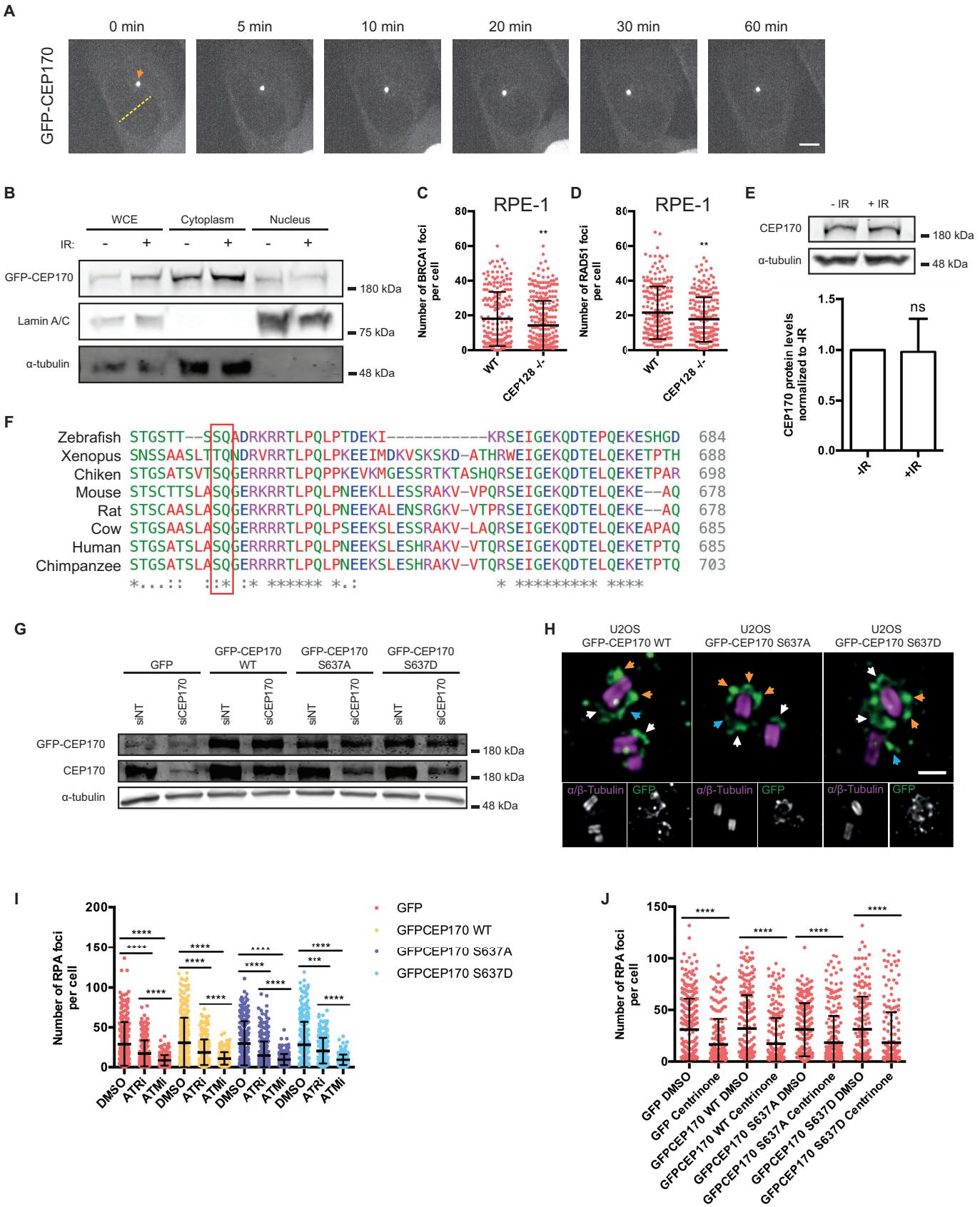

A
0 min
5 min
10 min
20 min
30 min
60 min
GFP-CEP170
B
WCE
Cytoplasm
Nucleus
IR:
GFP-CEP170
Lamin A/C
α-tubulin
180 kDa
75 kDa
48 kDa
C
RPE-1
Number of BRCA1 foci per cell
WT
CEP128 -/-
D
RPE-1
Number of RAD51 foci per cell
E
- IR
+ IR
CEP170
α-tubulin
CEP170 protein levels normalized to -IR
ns
F
Zebrafish STGSTT--SSQADRKRRTLPQLPTDEKI----------KRSEIGEKQDTEPQEKESHGD 684
Xenopus SNSSAASLTTQNDRVRRTLPQLPKEEIMDKVSKSKD-ATHRWEIGEKQDTELQEKETPTH 688
Chiken STGSATSVTSQGERRRRTLPQPPKEVKMGESSRTKTASHQRSEIGEKQDTELQEKETPAR 698
Mouse STSCTTSLASQGERKRRTLPQLPNEEKLLESSRAKV-VPQRSEIGEKQDTELQEKE--AQ 678
Rat STSCAASLASQGERKRRTLPQLPNEEKALENSRGKV-VTPRSEIGEKQDTELQEKE--AQ 678
Cow STGSAASLASQGERRRRTLPQLPSEEKSLESSRAKV-LAQRSEIGEKQDTELQEKEAPAQ 685
Human STGSATSLASQGERRRRTLPQLPNEEKSLESHRAKV-VTQRSEIGEKQDTELQEKETPTQ 685
Chimpanzee STGSATSLASQGERRRRTLPQLPNEEKSLESHRAKV-VTQRSEIGEKQDTELQEKETPTQ 703
G
GFP
GFP-CEP170 WT
GFP-CEP170 S637A
GFP-CEP170 S637D
siNT
siCEP170
GFP-CEP170
CEP170
α-tubulin
180 kDa
180 kDa
48 kDa
H
U2OS GFP-CEP170 WT
U2OS GFP-CEP170 S637A
U2OS GFP-CEP170 S637D
α/β-Tubulin
GFP
I
Number of RPA foci per cell
DMSO
ATRi
ATMi
GFP
GFPCEP170 WT
GFPCEP170 S637A
GFPCEP170 S637D
J
Number of RPA foci per cell
GFP DMSO
GFP Centrinone
GFPCEP170 WT DMSO
GFPCEP170 WT Centrinone
GFPCEP170 S637A DMSO
GFPCEP170 S637A Centrinone
GFPCEP170 S637D DMSO
GFPCEP170 S637D Centrinone

Figure EV3.

Figure EV4. Centrosomes or CEP170 loss hyper-sensitize to treatment with DNA damaging agents.

Representative images of clonogenic assays shown in Fig 4A–H.

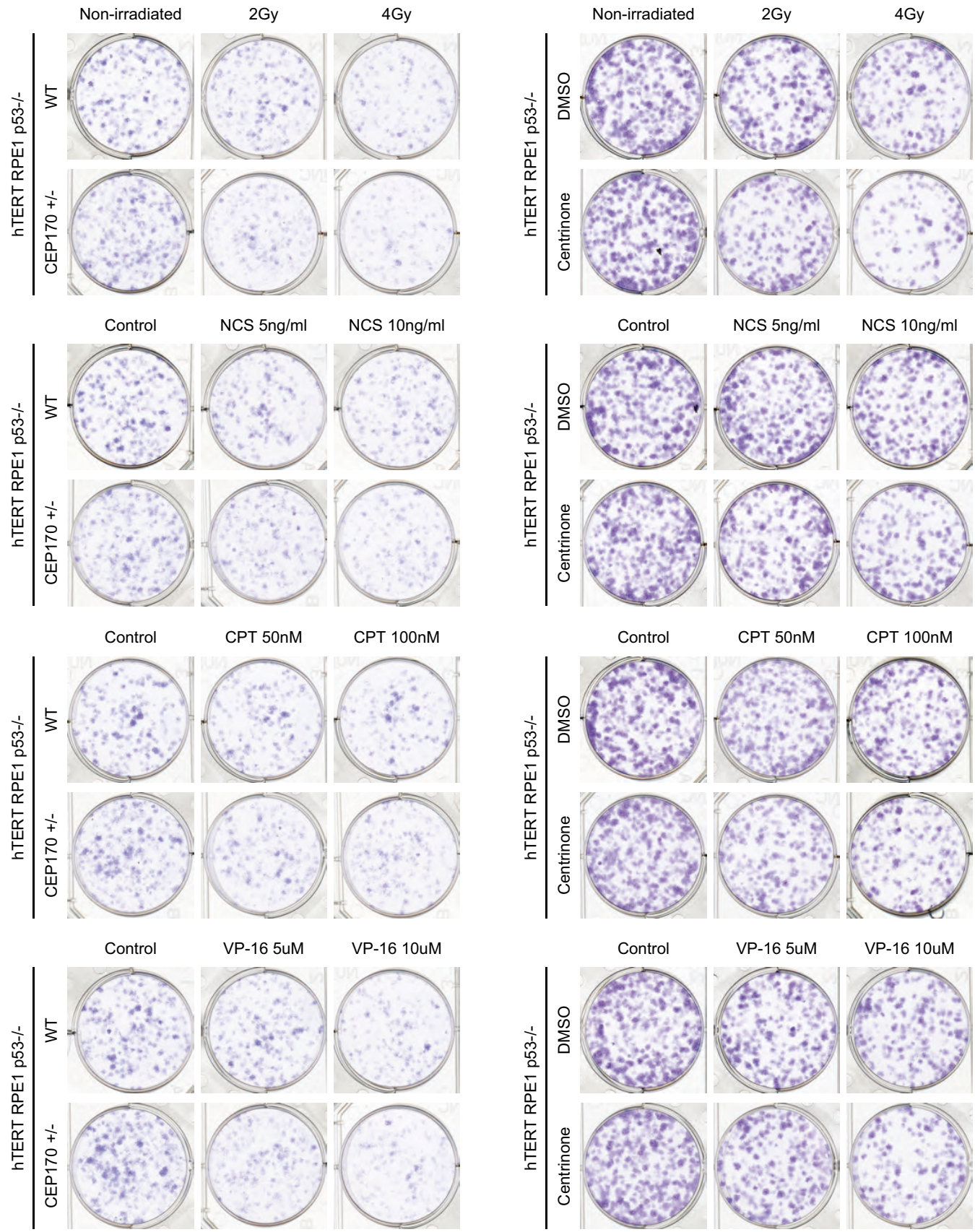


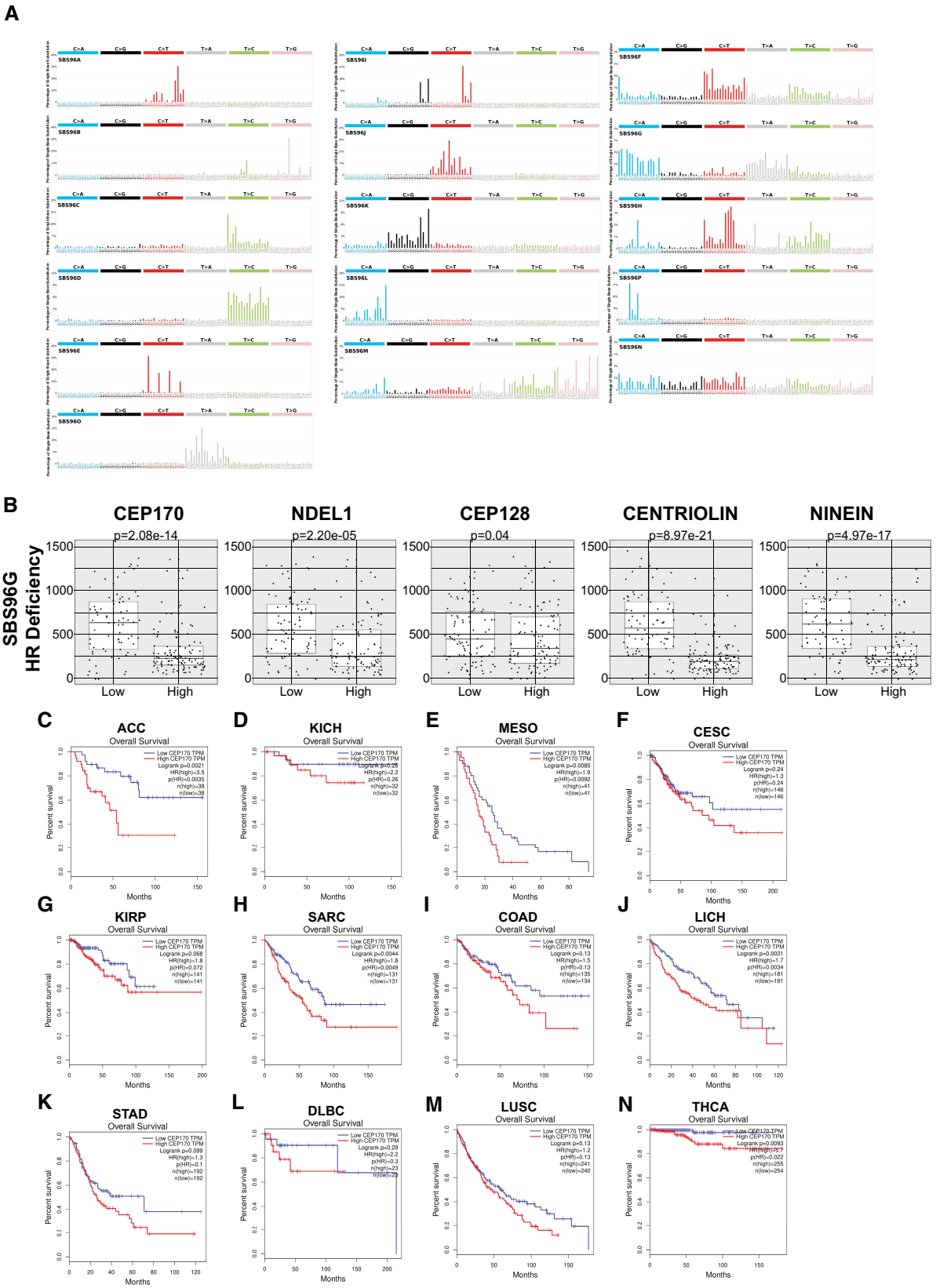
Figure EV4.

Figure EV5. Mutational signatures analyzed in tumor samples with low or high expression of CEP170.

A For each signature analyzed, the distribution of single base substitution considering the previous and following base is shown. Each signature presents a unique profile of mutations.

B Same as in Fig 5I but for samples at the lower quartile (low) or at the highest (high) of the indicated centriolar subdistal appendages proteins. The central band represents the median, the lower and upper hinges correspond to the first and third quartiles and the upper and lower whiskers extends from the hinge to the largest value no further than 1.5 inter-quartile range.

C–N Survival data in different cancer types with different CEP170 expressions were collected from the web-based tool GEPIA. Samples were split in two categories, the ones with an expression above the median (high; red lines) or below the median (low; blue lines). The number of individuals for each category is indicated in the top right corner of each chart. Overall survival of each category in each cancer type were plotted in Kaplan-Meyer graphs. Statistical significance was calculated using a Log-rank (Mantel–Cox) test and the *P*-values are included. Tumor types: (C) ACC, adrenocortical carcinoma. (D) KICH, kidney chromophobe. (E) MESO, mesothelioma. (F) CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma. (G) KIRP, kidney renal papillary cell carcinoma. (H) SARC, sarcoma. (I) COAD, colon adenocarcinoma. (J) LICH, liver hepatocellular carcinoma. (K) STAD, stomach adenocarcinoma. (L) DLBC, lymphoid neoplasm diffuse large B-cell lymphoma. (M) LUSC, lung squamous cell carcinoma. (N) THCA, thyroid carcinoma.


A
B
CEP170
p=2.08e-14
NDEL1
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CEP128
p=0.04
CENTRIOLIN
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NINEIN
p=4.97e-17
SBS96G
HR Deficiency
Low
High
C
ACC
D
KICH
E
MESO
F
CESC
G
KIRP
H
SARC
I
COAD
J
LICH
K
STAD
L
DLBC
M
LUSC
N
THCA
Overall Survival
Percent survival
Months

Figure EV5.