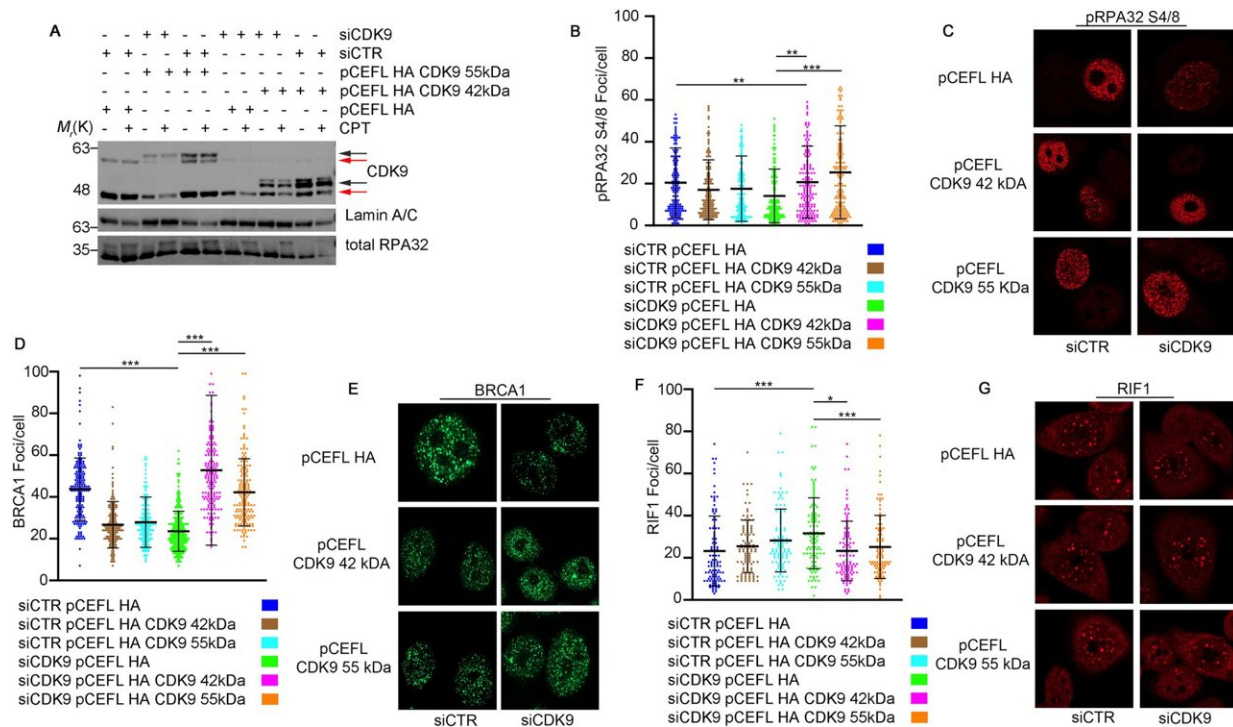


Cell division, DNA repair and cancer progression closely tied to CDK9 dysfunction

March 14 2024



CDK9 isoforms regulate DNA damage response. **A** Western blot analysis of CDK9 in HeLa cells transfected with siRNA CDK9 (siCDK9) or control (siCTR), and after 24 h transfected with the single CDK9 isoforms (pCEFLHA CDK9 55 kDa and pCEFL HA CDK9 42 kDa) or pCEFL HA control plasmid as indicated by + or -. Upon transfection, HeLa cells were treated with 1 μ M camptothecin (CPT), or not treated, for an additional 2 h. RPA32 was used as a DNA damage control, and Lamin A/C as a protein loading control. Red and black arrows indicate CDK9 endogenous and overexpressed protein isoforms, respectively. **B** Immunofluorescence analysis of HeLa cells transfected and treated as indicated in A; pRPA32 S4/8 foci number was measured by Fiji

software. More than 30 cells were analyzed for each condition in 3 independent experiments; data represent means \pm standard deviation. **C** Representative immunofluorescence image of pRPA32, from HeLa cells, transfected as in B, followed by incubation with CPT for two hours. **D** BRCA1 foci number of HeLa cells, transfected with siCDK9 for 24 h followed by transfection with indicated coding plasmid DNA, was measured by Fiji software. **E** Representative immunofluorescence image of BRCA1 of HeLa cells transfected and treated as in B. **F** RIF1 foci number was measured by Fiji software from HeLa cells transfected and treated as in B. For all immunofluorescence analyses more than 30 cells were analyzed for each condition in 3 independent experiments. **G** Representative immunofluorescence image of RIF1, from HeLa cells silenced with CDK9 for 24 h followed by differential overexpression of CDK9 42 or 55 kDa and treated with CPT for two hours. All graphs represent means \pm standard deviation. Data were subjected to one-way repeated measures ANOVA with Kruskal-Wallis post-test to compare all groups. Statistically significant differences are indicated with: ***P*-value

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