Supplementary Figure S3



sgDHX9

clePARP

V5 tag (RNase)

β-actin



Supplementary Figure S3.

A, qRT-PCR analysis of the direct irradiation response and replication-related genes comparing Scramble and sgDHX9 of H446 and H82 cells (n = 3). 36B4 gene was used as a reference. **B**, Immunofluorescence images of p-H2AX (red) staining of Scramble and sgDHX9 of H446 and H1048 cells. Scale bar = 50 μ m. C, Immunofluorescence images of DNA/RNA hybrid (red) staining of Scramble and sgDHX9 H196 cells, treated w/wo RNase H. Scale bar = $25 \mu m. D$, Immunoblot (IB) of the indicated proteins in Scramble and sgDHX9 of H82 and H1048 cells. E, Immunoblot (IB) of the indicated proteins in Scramble and sgDHX9 H82 cells, w/wo overexpression of RNase H1-V5. F, Immunoblot (IB) of the indicated proteins in H446 cells overexpressing 3xFlag-DHX9 (WT/K417R) or Flag-GFP, transfected with siCtrl or siDHX9-3'UTR. G, Relative cell number of H446 cells treated with the indicated siRNAs and expression vectors. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). H, Fluorescence intensity of DNA/RNA hybrid in H446 cells treated with the indicated siRNAs and expression vectors, was quantified (100 cells were counted per group). I, The percentage of stalled forks over the total number of different replication structures was measured (>150 labeled forks were counted per group, n = 3). J, GSEA analysis with C2 (curated) gene sets, based on RNAseq results of sgDHX9 versus Scramble cells. K, qRT-PCR analysis of the senescence-related genes comparing Scramble and sgDHX9 H196 cells (n = 3). 36B4 gene was used as a reference. Data represent mean \pm SEM. ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by unpaired Student's t test (A and K) and one-way ANOVA (G, H and I).