

## Supplementary Figures captions

### Figure S1

(A) DHP was probed by Western blotting in hTERT-immortalized retinal pigment epithelial cells (hTERT RPE-1) and in primary human dermal fibroblasts (HDF). Tubulin and Ponceau staining were used as loading control.

(B) HCT116 cells were transfected with control or two distinct anti-DHP siRNAs (siDHP-1 and siDHP-2). At the indicated times, viable cells were identified via trypan blue exclusion and counted. Mean viability is representative of three independent biological replicates. Error bars represent  $\pm$  S.D. Bottom panel: The efficiency of DHP knockdown was assessed by western blotting.

(C) AMOI cells were transfected with control or anti-DHP siRNAs. At the indicated times, viable cells were identified via trypan blue exclusion and counted. Mean viability is representative of three independent biological replicates. Error bars represent  $\pm$  S.D.

(D) HEK239T cells were transfected with shControl or shDHP-3. At the indicated times, viable cells were counted by trypan blue staining. Mean viability is representative of three independent biological replicates. Error bars represent  $\pm$  S.D.

(E) Colony-forming assay of HEK293T cells after transfection with control or anti-DHP shRNA (shDHP-3). A representative image is shown. An histogram represents the quantification of colony formation. Data shown are averages over three independent biological replicates with two technical replicates for each. Error bars represent  $\pm$  S.D. p-values were calculated using a regression model with Poisson distribution: \*\*\*  $P < 0.0001$ . Bottom panel: The efficiency of DHP knockdown was assessed by western blotting.

(F) Histogram representing the cell cycle distribution of HEK293T cells 72 hours after transfection with control or anti-DHP shRNA. Data shown are averages over three independent biological replicates. Error bars represent  $\pm$  S.D. p-values were calculated using a regression model with Poisson distribution: \*  $P < 0.05$ .

### Figure S2

(A) Upper panel: Experimental scheme. HEK293T cells transfected with control or anti-DHP shRNA were pulse-labeled with 20  $\mu$ M BrdU for 30 min, washed (W) and incubated for 6 hours in BrdU-free medium before analysis of the cell cycle distribution by two-dimensional (BrdU/DNA) flow cytometry. Right panel: Histogram representing the percentage of G1 cells that were in S phase (incorporating BrdU) 6 hours before the flow cytometry analysis. Data from three independent biological replicates are represented as mean  $\pm$  S.D. p-values were calculated using a regression model with Poisson distribution: \*\*  $P < 0.001$ .

(B) Graphic representation of replication track lengths in control and HEK293T cells for DHP using a shRNA molecule with a distinct target sequence in DHP. The bar dissecting the data points represents the median of 100 tracts length from one biological replicate. Differences between distributions were assessed with the Mann-Whitney rank sum test. p-values: \*\*\* < 0.0001.

(C) DHP knockdown HEK293T cells were subjected to subcellular fractionation and probed by western blotting with the indicated antibodies. *Tubulin and histone H3* were used as *loading controls* for the cytoplasmic and chromatin *fractions*, respectively. One representative experiment is shown from two biological replicates.

(D) DHP-depleted U-2 OS cells were subjected to subcellular fractionation and probed by western blotting with the indicated antibodies. *Tubulin and histone H3* were used as *loading controls* for the cytoplasmic and chromatin *fractions*, respectively. One representative experiment is shown from two biological replicates.

(E) Whole-cell extracts from control and DHP knockdown HEK293T cells (shDHP-4) were analyzed by western blotting with the indicated antibodies. One representative experiment is shown from two biological replicates.

(F) Quantification of the percentage of RPA32 foci positive cells in a population of 100 cells of DHP-depleted HEK293T cells (shDHP-4). Data from three independent biological replicates are represented as mean +/- S.D. p-values were calculated using a regression model with Poisson distribution: \*\*\*  $P < 0.0001$ .

### Figure S3

(A) Analysis of dNTP concentrations in control and DHP knockdown HEK293T cells. Data from three independent biological replicates, with three technical replicates for each, are represented as mean  $\pm$  S.D. Cellular dNTPs were measured by HIV-RT based dNTP assay (Diamond TL et al, 2004). p-values were calculated using a regression model with Poisson distribution: \*  $P < 0.05$ , ns: not significant.

(B) Analysis of dNTP concentrations in control and DHP knockdown U-2 OS cells. Data from three independent biological replicates, with three technical replicates for each, are represented as mean  $\pm$  S.D. p-values were calculated using a regression model with Poisson distribution: \*\*\*  $P < 0.0001$ , \*  $P < 0.05$ , ns: not significant.

(C) Control and DHP knockdown HEK293T cells were supplemented with nucleosides and incubated for 18 hours before DNA fiber analysis of the length of CldU labeled replication tracks, in  $\mu\text{m}$  (y axis). The bar dissecting the data points represents the median of 100 tracts length from one biological replicate. Differences between distributions were assessed with the Mann-Whitney rank sum test. p-values: \*\*\*  $< 0.0001$ .

(D) Control and DHP knockdown U-2 OS cells (siDHP-1) were supplemented with nucleosides and incubated for 18 hours before western blot analysis with the indicated antibodies. Ponceau staining was used as loading control.

(E) Western blot analysis with the indicated antibodies of whole cell extracts from U-2 OS transfected with anti-DHP and anti-UPP1 siRNAs. One representative experiment is shown from two biological replicates.

#### **Figure S4**

(A) Replicate of chromatin transfer as described in Figure 4D. Replication products were resolved by 1 % alkaline agarose gel electrophoresis and revealed by autoradiography. Lanes 1-4: Mock treated extracts; Lanes 5-8: incubation in the first extract was performed in the presence of 7.5 mM DHU. Lanes 10-12 serve as positive controls: after 30 min incubation in the first extract, DNA synthesis was blocked with aphidicolin (100 ng/ $\mu$ l). Right panel: Histogram representing the quantification of the gel by image J of replication products (arbitrary unit).

(B) Purified His-tagged DHP was resolved by SDS/PAGE and stained with Coomassie (lane 2). Size marker (lane 1). \* non-specific band.

(C) Western blot analysis with the indicated antibodies of U-2 OS whole-cell extracts treated with dihydromyricetin for 12 hr at the indicated concentrations. One representative experiment is shown from three biological replicates.

(D) Western blot analysis with the indicated antibodies of whole cell extracts from U-2 OS transfected with anti-UPP1 siRNA and treated or not with 20  $\mu$ M of dihydromyricetin for 24 hr. One representative experiment is shown from two biological replicates.

## Figure S5

(A) DHP knockdown HEK293T cells were subjected to subcellular fractionation and probed by western blotting with the indicated antibodies. *Tubulin* and *histone H3* were used as *loading controls* for the cytoplasmic and chromatin *fractions*, respectively.

(B) Histogram representing the quantification of DPC levels of HEK293T cells transfected with control or two anti-DHP shRNAs (shDHP-3 and shDHP-4) normalized to total DNA. Two independent biological replicates are represented. p-values were calculated using a regression model with Poisson distribution: \*\*\*  $P < 0.0001$ .

(C) Graphic representation of replication track lengths in U-2 OS cells treated or not with 0.1  $\mu\text{M}$  Aphidicolin for 30 min. The bar dissecting the data points represents the median of 100 tracts length from one biological replicate. Differences between distributions were assessed with the Mann-Whitney rank sum test. p-values: \*\*\*  $< 0.0001$ .

(D) Total DPC levels in U-2 OS cells treated or not with 0.1  $\mu\text{M}$  Aphidicolin for 30 min visualized by silver staining. Right panel: Histogram representing the quantification of DPC levels normalized to total DNA amount by image J. Three independent biological replicates are averaged in the bar graphs. Error bars represent  $\pm$  S.D. p-values were calculated using a regression model with Poisson distribution: ns; not significant. Bottom panel: Western blot analysis of crosslinked DNA polymerase  $\eta$  in total DPC extracts from the experiment above. One representative experiment is shown from three biological replicates.

**Figure S6**

(A) U-2 OS cells were transfected with the indicated siRNAs and exposed to increasing concentrations of dihydromyricetin for two days. Cell viability was estimated using Cell Titer-Glo assay. Mean viability is representative of experiments performed in triplicate. Error bars represent +/- S.E.M. Right panel: the efficiency of XPA, SPRTN and ERCC5/XPG knockdown was assessed by western blotting.

(B) HEK293T cells were transfected with control or anti-FANCM shRNA and, after 36 hours, exposed to increasing doses of dihydromyricetin for 48 hours. Cell viability was assessed by Cell Titer-Glo. Mean viability is representative of experiments performed in duplicate. Error bars represent +/- S.E.M.

(C) HEK293 FANCM WT and FANCM K117R cells were exposed to increasing doses of dihydromyricetin for 48 hours. Cell viability assessed by Cell Titer-Glo. Mean viability is representative of experiments performed in triplicate. Error bars represent +/- S.D.