Expanded View Figures

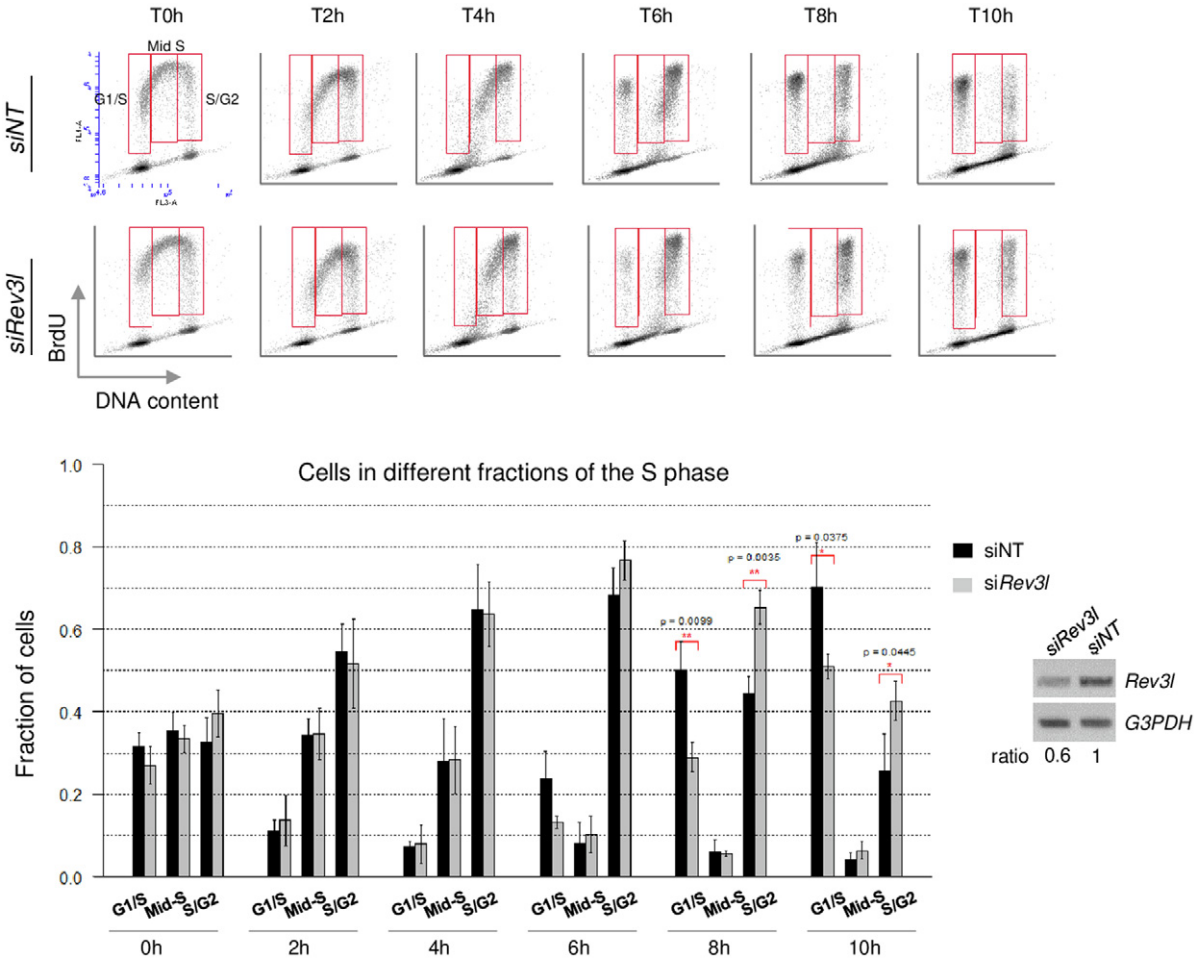

Figure EV1. S-phase progression is impaired in human cells after down-regulation of REV3L.

Forty-two hours after transfection with non-targeting (NT) siRNA or *Rev3l* siRNA, HeLa cells were pulse-labeled with BrdU prior to harvest and analyzed by flow cytometry at different time points (top panel). Analysis was focused on S-phase divided into three parts: G1/early S, middle S, and late S/G2 BrdU^{+} cells. Histograms represent the percentage of cells in G1/early and late S/G2-phase after the BrdU pulse (bottom panel). Error bars indicate standard error of the mean from three independent experiments (Student's *t*-test). RT–PCR was performed to verify the down-regulation of *Rev3l* mRNA level in HeLa cells.

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Figure EV2. Loss of REV3L disrupts replication timing in specific genomic loci.

A Cells were pulse-labeled with BrdU for 1.5 h and sorted by flow cytometry in two fractions, S1 and S2, corresponding to early and late S-phase fractions, respectively. Neo-synthesized DNA was immunoprecipitated with BrdU antibodies. Early and late neo-synthesized DNAs were labeled with Cy3 and Cy5, respectively, and hybridized on microarrays. After processing analysis, replication-timing profiles were obtained. Shown are the zoomed microarray profiles of the timing of replication on chromosome 1 (136.6–143.6 Mb), chromosome 5 (127.2–136.4 Mb), and chromosome 9 (44.8–54.1 Mb) from *Rev3l*$^{+/+}$ and *Rev3l*$^{-/-}$ MEFs overlaid. Black lines represent replication timing from *Rev3l*$^{+/+}$ MEFs, and gray lines represent *Rev3l*$^{-/-}$ MEFs. For each group, replication timing at early passage (p5 or p7) and late passage (p60) was analyzed and shown a great conservation of replication timing over serial passages (double line on zoomed microarray profiles for each cell line, see corresponding arrow).

B Fraction of advanced and delayed regions in the disturbed replication timing in *Rev3l*$^{-/-}$ MEFs. The proportions were calculated either in the percentage of numbers of advanced and delayed regions, or the size of advanced and delayed regions (in base pairs).

C HeLa cells were transfected with non-targeting siRNA (siNT) or siRNA against *Rev3l;* then, 60 h later cells were pulse-labeled with BrdU for 1.5 h and sorted by flow cytometry in two fractions, S1 and S2, corresponding to early and late S-phase fractions, respectively. Neo-synthesized DNA was immunoprecipitated with BrdU antibodies. Early and late neo-synthesized DNAs were labeled with Cy3 and Cy5, respectively, and hybridized on microarrays. After processing analysis with the START-R software, replication-timing profiles were obtained. Shown are the zoomed microarray profiles of the timing of replication on chromosome 1 (47–67 Mb), chromosome 3 (5–37 Mb), and chromosome 17 (47–67 Mb) from HeLa *siNT* and *siRev3l* overlaid. Blue lines represent replication timing from HeLa *siNT*, and red lines represent replication timing from HeLa *siRev3l*. Genomic regions detected significantly different by START-R are indicated by a pink line ($P < 0.01$).

D Fraction of advanced and delayed regions in the disturbed replication timing in HeLa *siRev3l*. The proportions were calculated either in the percentage of numbers of advanced and delayed regions or in the size of advanced and delayed regions (in base pairs).

E Boxplot of coverage of the disturbed regions in each category (early, mid, late, TTR). Bar in boxplot represents the median, and red points represent the average. The limit of the boxes corresponds to the 0.25–0.75 quartiles with whiskers extending to the maximum value of 1.5 times the interquartile range. Graphs show data from one biological experiment.

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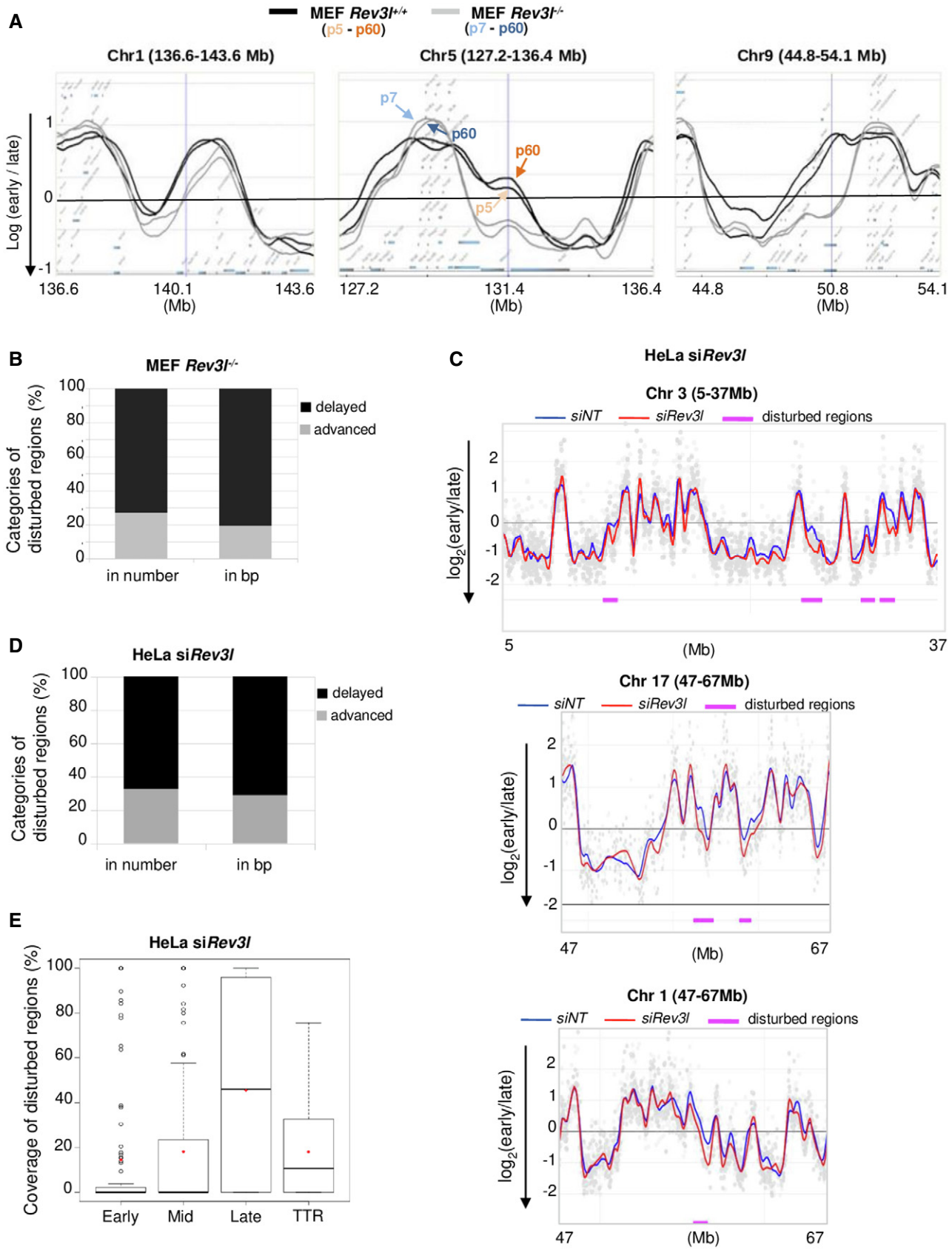

Figure EV2.

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Figure EV3. REV3L interacts with heterochromatin components.

A Diagram of the mouse *Rev3l* 3x-FLAG knock-in allele. The wild-type (WT) locus is shown at the top (left panel). A green rectangle shows the first exon with the initiator ATG sequence. The gray line represents chromosomal sequence. The middle diagram shows the targeted locus after homologous recombination. The neomycin-positive selection cassette (neo) is shown in gray. The cassette was excised by breeding mosaic animals with C57BL/6 Cre deleter mice, to produce the 3x-FLAG-tagged knock-in allele shown at bottom. Example of Southern blot analysis of the knock-in locus. Genomic DNA of the tested animals (number 115503 shown here) was compared with C57BL/6 wild-type genomic DNA (WT). *Bgl*II digested DNA was blotted on a nylon membrane and hybridized with the external 3′ probe with the position shown at the left. Restriction fragments of 9.4 and 4.2 kb were observed for the wild-type, and knock-in locus, respectively. Genomic DNA was further analyzed extensively and confirmed by specific PCR assays and complete DNA sequencing as described in the Materials and Methods (right panel).

B Total proteins were extracted from MEFs or MEFs expressing endogenous Flag-REV3L; then, REV3L protein was detected by immunoblotting using anti-Flag (M2) antibody. Specific signal above 250 kDa is detected only in MEF Flag-REV3L.

C Specificity of Flag-REV3L detection was confirmed by down-regulating the REV3L protein. MEF Flag-REV3L was transfected with non-targeting siRNA (NT) or siRNA targeting *mRev3l*. Seventy-two hours later, cells were lysed; then, proteins were analyzed by immunoblotting using anti-Flag (M2) antibody or anti-p125 antibody as loading control. The experiment was repeated twice (Exp#1 and #2).

D Asynchronous MEFs expressing Flag-tagged REV3L were subjected to *in situ* proximity ligation assay (PLA) to test the interactions REV3L-REV7 and REV3L-HP1α as indicated in Fig 5B. Representative images from each PLA condition with controls are shown. Scale bar: 20 μm.

E Asynchronous MEFs expressing Flag-tagged REV3L were subjected to PLA to test the interactions REV3L-H4K20me3, REV3L-H3K9me3, REV3L-H3K27me3, REV3L-H3K4me3, and REV3L-H3K9ac as indicated in Fig 5C. Representative images from PLA REV3L-H4K20me3 and REV3L-H3K9ac with their controls are shown. Note the high background for H3K9ac control. Scale bar: 20 μm.

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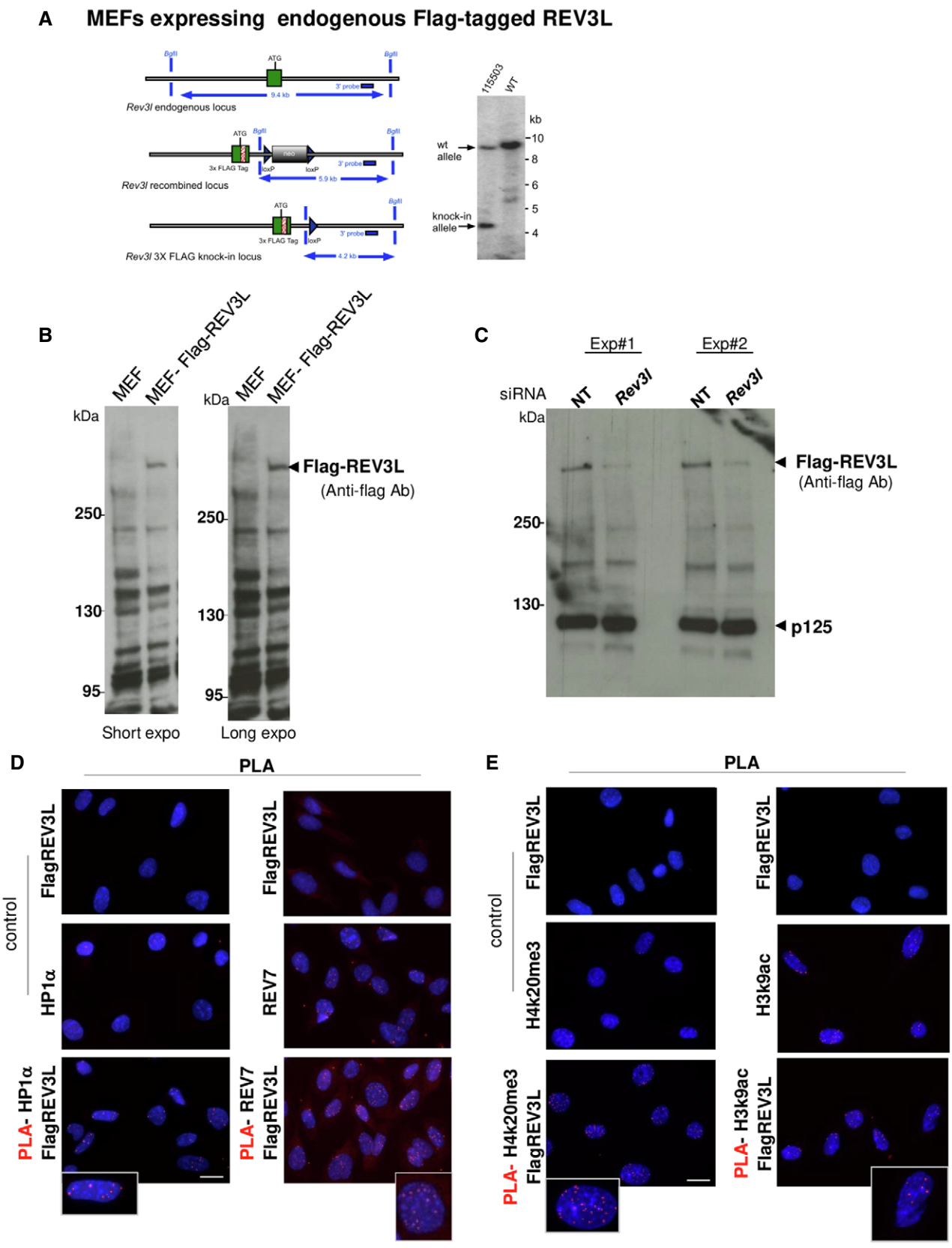

Figure EV3.

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Figure EV4. PxVxL motif targets REV3L to pericentromeric heterochromatin through a direct interaction with HP1.

A Wild-type cells and MEFs lacking Suv39h (*Suv39h* dn) were transiently transfected with GFP-REV3L$^{761–1,029}$ construct then fixed with 4% formaldehyde after 0.5% Triton X-100 pre-extraction. The distribution of GFP-REV3L$^{761–1,029}$ was detected by autofluorescence, HP1α immunodetection was processed using specific antibodies (red), and nuclei were visualized by DAPI staining. Scale bar = 10 μm.

B Wild-type MEFs were transiently transfected with GFP-REV3L$^{761–1,029}$ construct and treated or not for 24 h with trichostatin A (TSA). Cells were released or not with fresh medium during 6 h and fixed with 4% formaldehyde after 0.5% Triton X-100 pre-extraction. The distribution of GFP-REV3L$^{761–1,029}$ was detected by autofluorescence, HP1α was immunodetected using antibodies (red) and nuclei were visualized using DAPI staining. Scale bar = 10 μm.

C Sequence alignment of REV3L protein among different species containing the PxVxL motif with canonical residues shown in blue.

D Schematic representation of REV3L$^{2,732–3,130}$ construct. MEF cells were transiently transfected with GFP-REV3L$^{2,732–3,130}$ or GFP-REV3L$^{761–1,029}$ constructs and fixed with 4% formaldehyde. The distribution of GFP-REV3L constructs was detected by autofluorescence and nuclei with DAPI staining. Scale bar = 10 μm.

E 293T cells were co-transfected with F-H-REV3L full-length or empty vector and V5-HP1γ, mutant IY165 168EE V5-HP1γ, mutant V32 M IY165 168EE V5-HP1γ or empty vector. Forty-eight hours after transfection, cell lysates were made and used for immunoprecipitation with V5 antibody. After electrophoresis, samples were immunoblotted with anti-HA, anti-HP1γ, or anti-α–Tubulin as indicated.

F GST, GST-REV3L$^{700–900}$, mutant V802D GST-REV3L$^{700–900}$, His-XPA-FLAG, His-HP1α, and His-HP1γ were purified from *E. coli* and stained with Coomassie Brilliant Blue. These were used with glutathione beads for GST pulldown experiments. After electrophoresis, samples were immunoblotted with anti-His or anti-GST as indicated.

G Images from REV3L-HP1α PLA experiment described in EV3D were analyzed for the localization of PLA signals in the nucleus by distinguishing PLA foci In/around chromocenters and PLA foci out of chromocenters using ImageJ tools. Chromocenters were identified by the DAPI density. One hundred fifteen nuclei have been analyzed. Red lines indicate the mean values ± SD.

Source data are available online for this figure.

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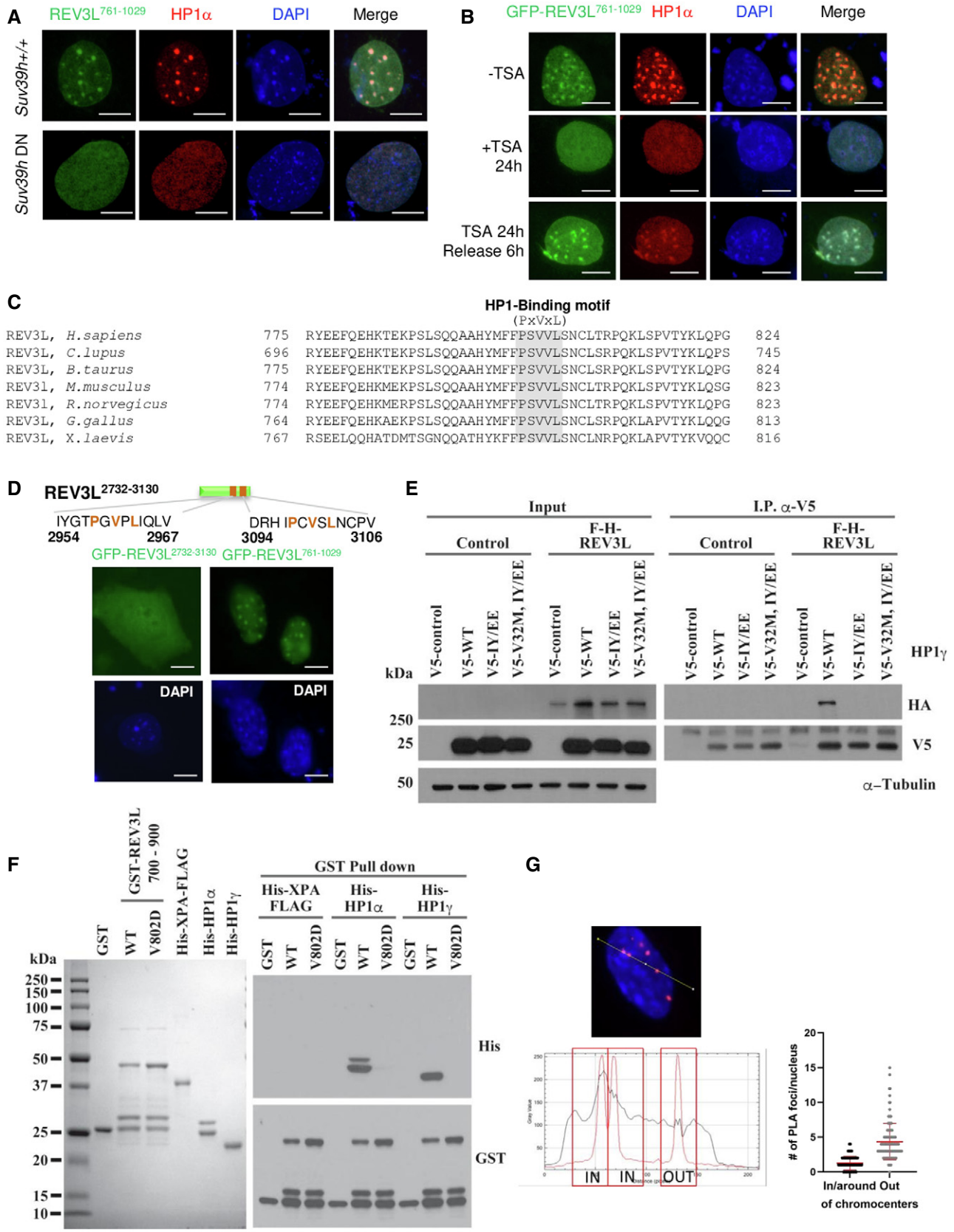

Figure EV4.

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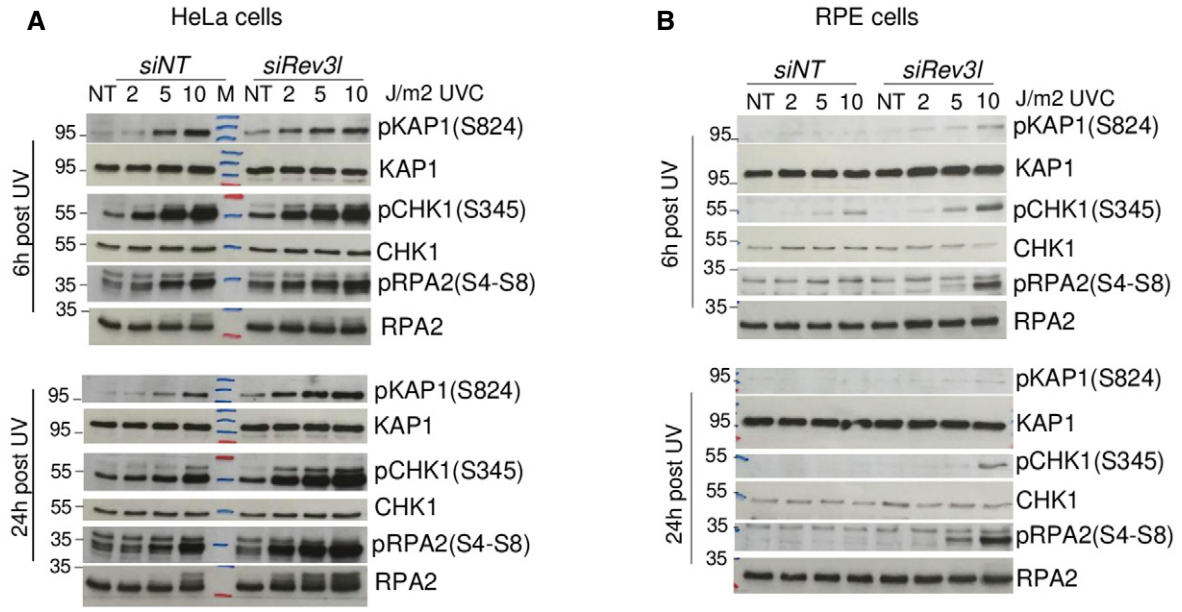

Figure EV5. REV3L loss impacts on heterochromatin-associated DSB repair in human cells.

A, B HeLa cells (A) or RPE cells (B) were transfected with non-targeting siRNA (siNT) or siRNA against *Rev3l*; then, 72 h later cells were UV-irradiated at the indicated doses and harvested 6 or 24 h post-irradiation. Cell lysates were analyzed by immunoblotting with indicated antibodies.

Source data are available online for this figure.

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