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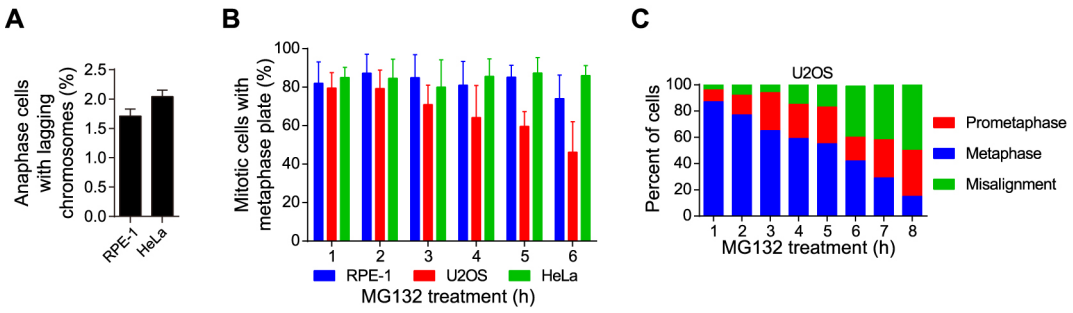
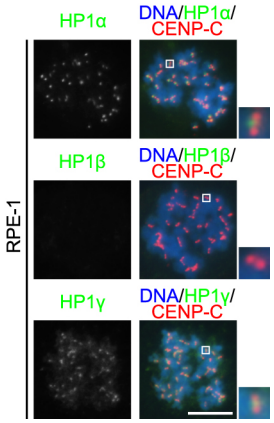


Appendix Figure S1. Comparison of three cell lines for chromosomal stability. A HeLa cell line behaves like RPE-1 cells with regard to few segregation errors in anaphase and the proper maintenance of metaphase chromosome bi-orientation.

A RPE-1 and HeLa cells were fixed and stained with ACA antibodies and DAPI. The percentage of cells with lagging chromosomes was determined in 200 anaphase cells. Means and ranges are shown (n = 2).

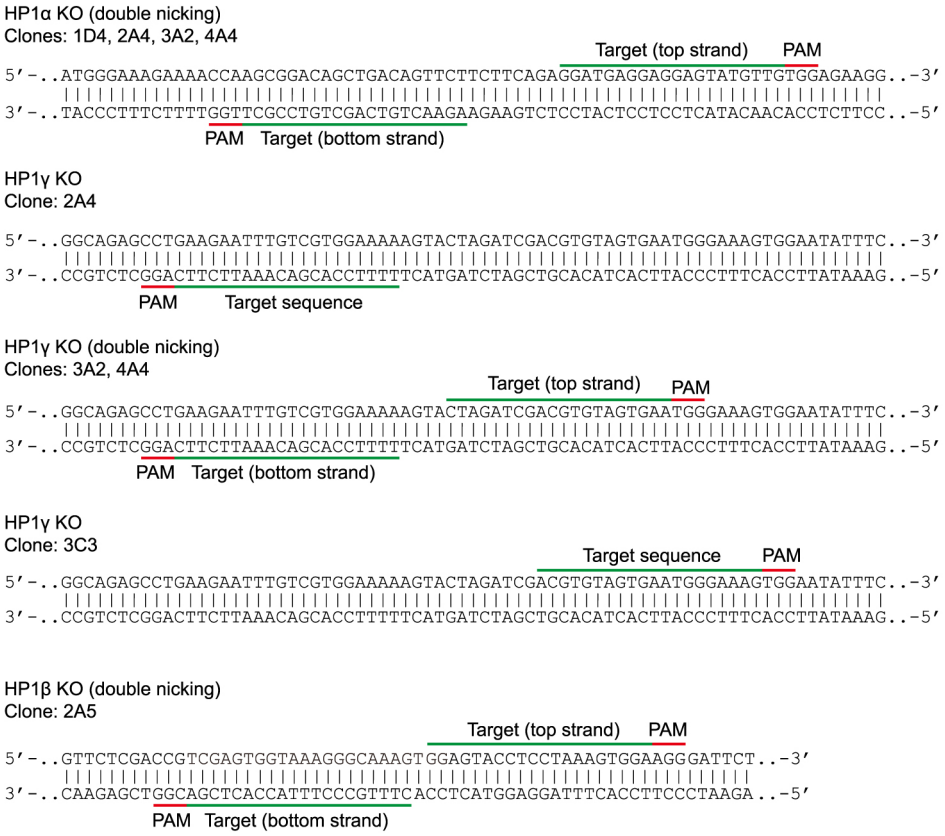
B RPE-1, U2OS and HeLa cells were exposed to MG132, then fixed at the indicated time points for DNA staining. The percentage of mitotic cells with standard metaphase plate was determined in around 300 cells. Means and SDs are shown (n = 3).

C U2OS cells were exposed to MG132, then fixed at the indicated time points for DNA staining. The percentage of mitotic cells in prometaphase, metaphase, and metaphase some misaligned chromosomes, was determined in around 100 cells.

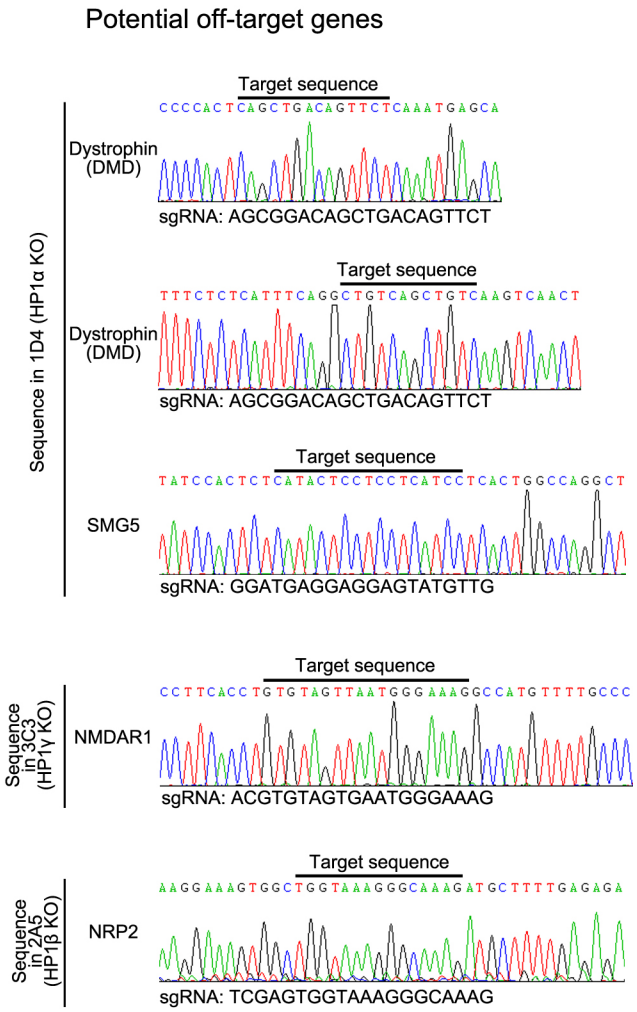


Appendix Figure S2. Localization of HP1 proteins on mitotic chromosomes of RPE-1 cells. RPE-1 cells were treated with nocodazole for 3 h. Mitotic chromosome spreads were immunostained with the indicated antibodies. Scale bar, 10 μm.

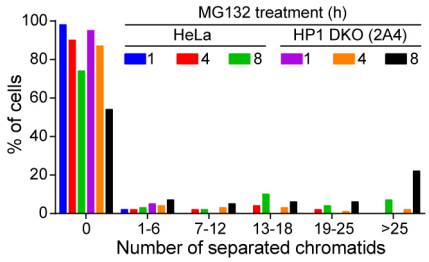
Appendix Figure S3



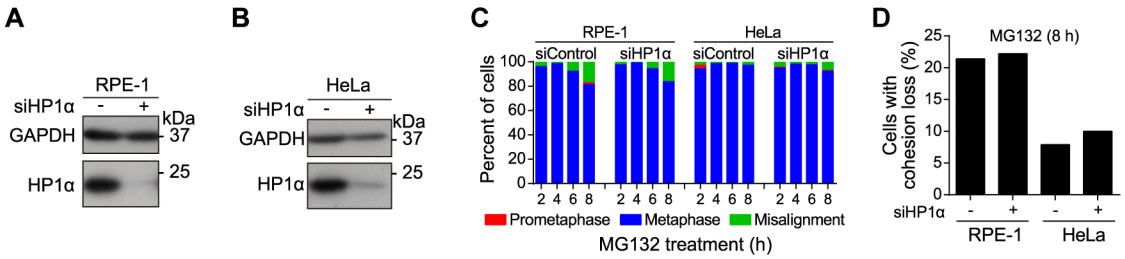
Appendix Figure S3. The sgRNAs with target HP1 DNA sequences preceding a 5'-NGG PAM.



Appendix Figure S4. Genomic DNA sequencing of potential off-target genes in HeLa-derived HP1 KO clones. The genomic DNA fragments were PCR amplified and sequenced to confirm that these genes are not disrupted.



Appendix Figure S5. Distribution in the number of separated chromatids in metaphase HeLa and HP1 DKO clone 2A4 cells. HeLa and HP1 DKO clone 2A4 cells were exposed to MG132 for 1 h, 4 h and 8 h. Using mitotic chromosome spreads, the percentage of cells with the indicated number of separated chromatids was determined in around 100 cells.



Appendix Figure S6. HP1α knockdown by siRNA does not compromise sister-chromatid cohesion in RPE-1 and HeLa cells.

A, B Asynchronous RPE-1 (A) and HeLa (B) cells transfected the indicated siRNAs were immunoblotted;

C RPE-1 and HeLa cells transfected the indicated siRNAs were exposed to MG132, fixed at the indicated time points for DNA staining, and quantified in around 100 cells. The percentage of mitotic cells in prometaphase, metaphase, and metaphase with some misaligned chromosomes, was determined in around 100 cells.

D RPE-1 and HeLa cells transfected the indicated siRNAs were exposed to MG132 for 8 h, then mitotic cells were used to prepare chromosome spreads. The percentage of cells with cohesion loss was determined in around 100 cells.