

**Figure S1: HIPS2 differentiation and *CENP-A* levels.** A. Cell cycle analysis of undifferentiated, day 4 and day 7 differentiated HIPS2 compared to BJ cells (n=3 replicates each). B. Quantitative Real-time PCR for *CENP-A* mRNA expression in HIPS2 with differentiation up to day 7 (n=3 replicates).

**Figure S2: Characterization of *CENP-A* knockdown HSF-6.** A. Morphological appearance of BJ control and *CENP-A* knockdown cells after 5 days starting from equal seeding densities. C. Quantitative Real-time PCR for pluripotency genes *OCT4* (n=4 replicates), *NANOG* (n=7 replicates) and *SOX2* (n=4 replicates) in *CENP-A* knockdown HSF-6 compared to control. All values are normalized against *GAPDH*. D. *SSEA4* expression in control and *CENP-A* knockdown cells measured by flow cytometry after 5 days of selection with hygromycin (n=3).

**Figure S3: Depletion of *CENP-A* in HSF-1 hESCs.** A. Annexin V staining of hPSCs (HSF-1) (n=3 replicates). B. *SSEA4* staining in Control and *CENP-A* knockdown HSF-1 cells under self-renewing conditions (n=3 replicates). C. Annexin V staining of Control and *CENP-A* knockdown HSF-1 cells after 4 days of differentiation with Retinoic Acid (n=3). D. Apoptosis in 4 days differentiated Control and *CENP-A* knockdown HSF-1 cells before and after treatment with 30  $\mu$ M of Pifithrin (n=3 replicates).





