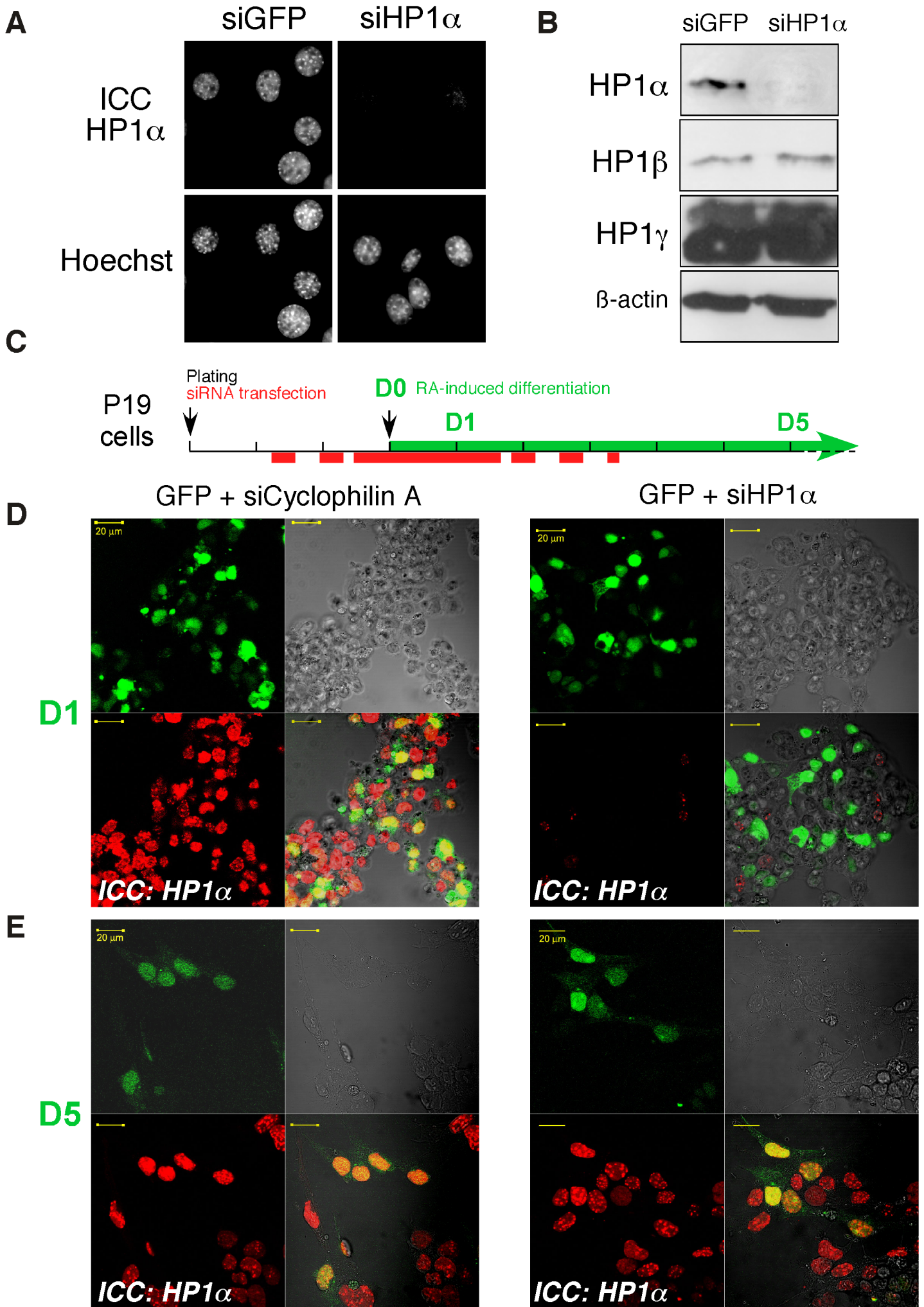


Supplementary data #S3



S3. Validation of HP1 α knock down. (A, B) NIH 3T3 cells were transfected with control siRNA (siGFP) or siRNA against HP1 α (siHP1 α) at 30nM for 72 hours. (A) Immunocytochemistry (ICC) was performed with an antibody against HP1 α and nuclear staining with Hoechst. (B) Immunoblots were performed on total cell extracts with antibodies against either HP1 α , β , γ or β -actin for loading. (C) **Schematic of the experimental design for siRNA transfection.** The red (dotted) line represents the (predicted) timing of HP1 α knock-down efficiency, evaluated in (D, D1) and (E, D5). siRNA have to be transfected 3 days before the induction of differentiation in order to produce a maximum of knock-down at D1 of RA treatment. The green arrow represents the progression through differentiation. (D) P19 cells were co-transfected with GFP and 30nM of siRNA against cyclophilin A (siCycA) as control or siHP1 α , 70 hours before induction of differentiation. Cells were harvested 1 day after and ICC for HP1 α was performed. After siCycA transfection, all cells are labeled with an HP1 α antibody (in red), while only a few cells remained HP1 α positive after siHP1 α transfection. It is noteworthy that a large population of non GFP positive cells were also lacking HP1 α , showing the high percentage of siRNA transfection. (E) P19 cells were transfected as in D and allowed to differentiate for 5 days before HP1 α ICC. All nuclei express HP1 α again at this period of time.