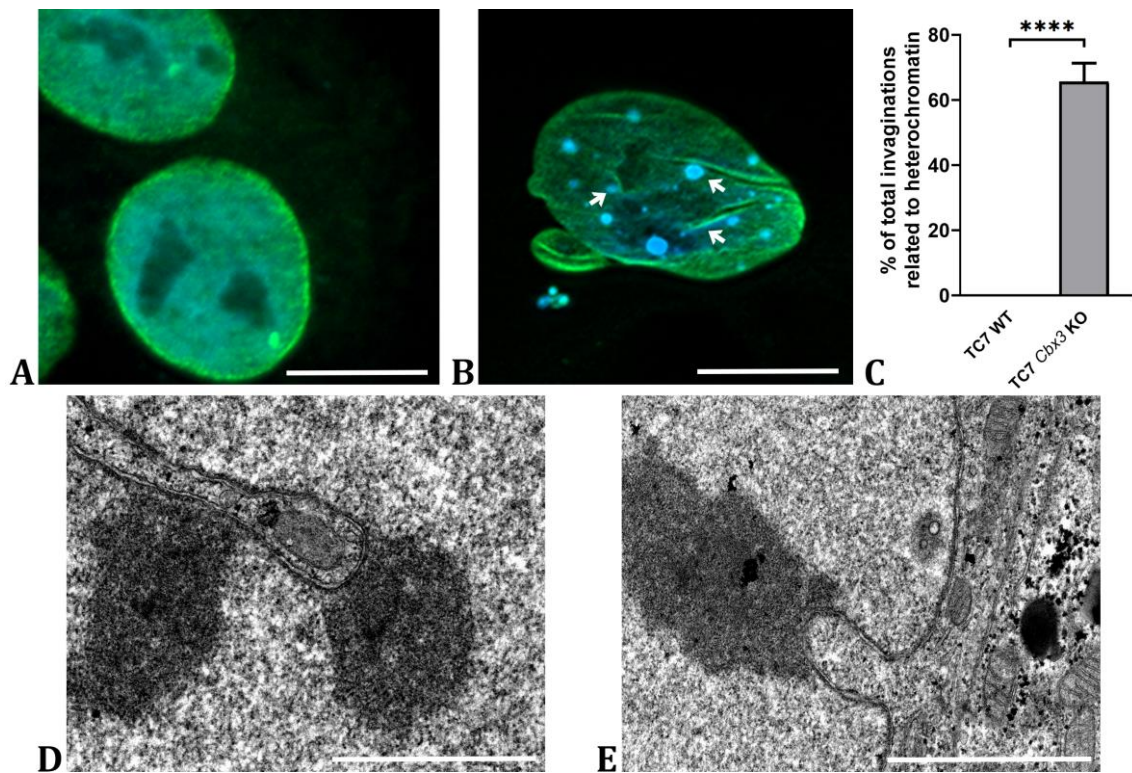
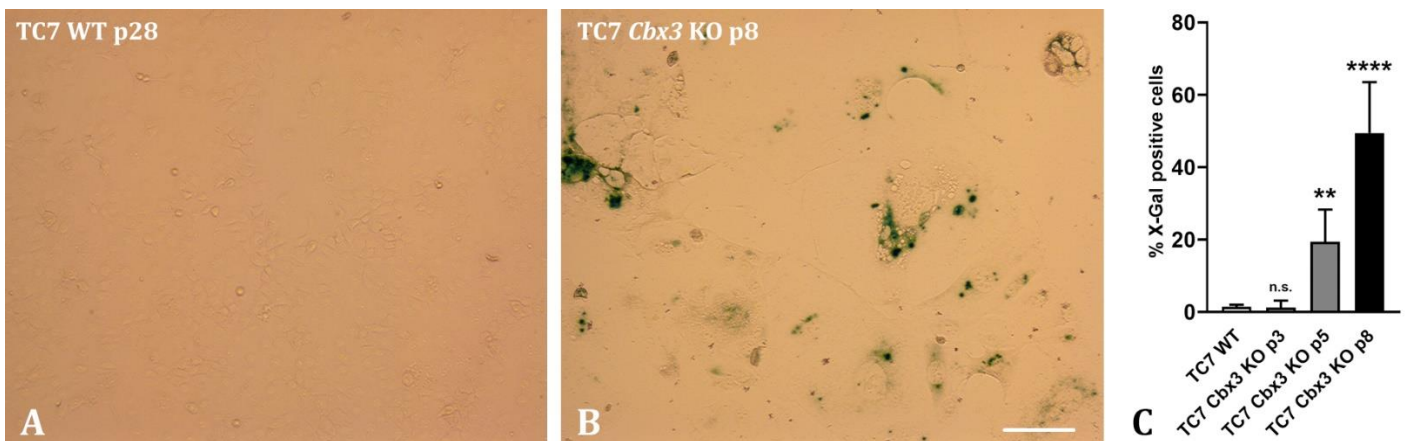


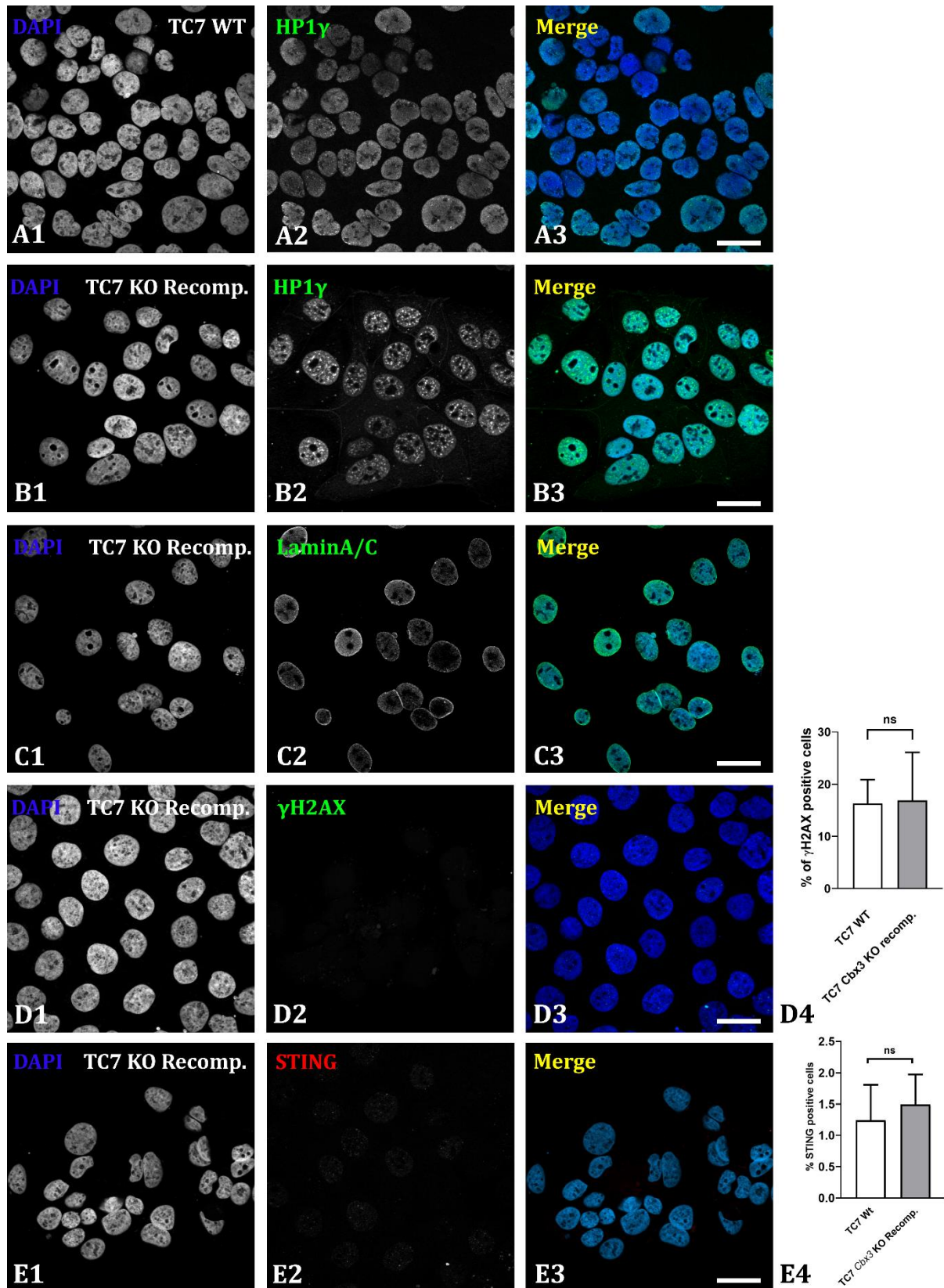
Supplementary Figure S1. Efficiency of *Cbx3* Knockdown in TC7 cells: (A-F) Immunofluorescence with anti-HP1γ antibody showing that the protein is abundant in the nucleus of WT cells (B) while it is undetectable in *Cbx3* KO cells (E). Images are representative of $n = 6$ clones (generated by 1 sgRNA) with 3 independent experiments/clone. Scale bar: 25 μm



Supplementary Figure S2. Quantitative analysis of the number of nuclear envelope invaginations associated with heterochromatin. (A,B) Immunofluorescence of laminA/C showing envelope invaginations that tend to associate with heterochromatin foci, labeled by DAPI (B, arrows). While the nuclear envelope is shown to be uniform and without invaginations in TC7 WT cells (A). (D,E) Electron microscopy showing nuclear envelope invaginations in the TC7 *Cbx3* KO cells that associate with heterochromatin foci. (C) Quantitative analysis of the number of invaginations in which association with heterochromatin was observed. $n = 6$ clones (generated by 1 sgRNA), 3 independent experiments/clone (t-test and non-parametric, two-tailed P value). Scale bar: A,B = 25 μm ; D,E = 1 μm .



Supplementary Figure S3. Study of the viability of TC7 *Cbx3* KO cells by analyzing the incorporation of 5-bromo-4-chloro-3-indoyl β -D-galactopyranoside (X-Gal). X-Gal staining (greenish labeling) in (A) TC7 WT cells with no positive cells detectable whereas in *Cbx3* KO cells (B) a signal was detected, along with enlargement of the cell size (C) Quantitative analysis of the number of X-Gal positive cells in TC7 WT and TC7 *Cbx3* KO cells. WT cells show less than 1% of positive cells (passage 28), with a comparable level of X-Gal staining in TC7 *Cbx3* KO cells at passage 3. However, at passage 5, the percentage of positive cells increased considerably up to 20%, reaching a maximum of 50% at passage 8. At this point, cells lacking HP1 γ stopped dividing. One-way ANOVA. ** $p < 0.01$; **** $p < 0.0001$. $n = 6$ clones (generated by 1 sgRNA), 3 independent experiments/clone. Scale bar: 50 μm .



Supplementary Figure S4. Immunofluorescence analysis of the phenotype of TC7 *Cbx3* KO cells upon stable reintroduction of HP1 γ expression: Immunofluorescences were performed as follow: (A1–A3) TC7 WT cells with Dapi-/HP1 γ antibody; (B1–E3) TC7 *Cbx3* KO cells reconstituted with HP1 γ with the corresponding antibody: (B1–B3) Dapi/anti-HP1 γ , (C1–C3) Dapi/lamina/C, (D1–D3) Dapi/ γ H2AX. (D4) Quantification positive cells for γ H2AX signal in WT and TC7 *Cbx3* KO cells reconstituted with HP1 γ . (E1/E3) Dapi/STING. (E4) Quantification positive cells for STING signal in WT and TC7 *Cbx3* KO cells reconstituted with HP1 γ . $n = 3$ clones (generated by 1 sgRNA), 3 independent experiments/clone (t -test and non-parametric, two-tailed P value). Scale bar: 25 μ m.