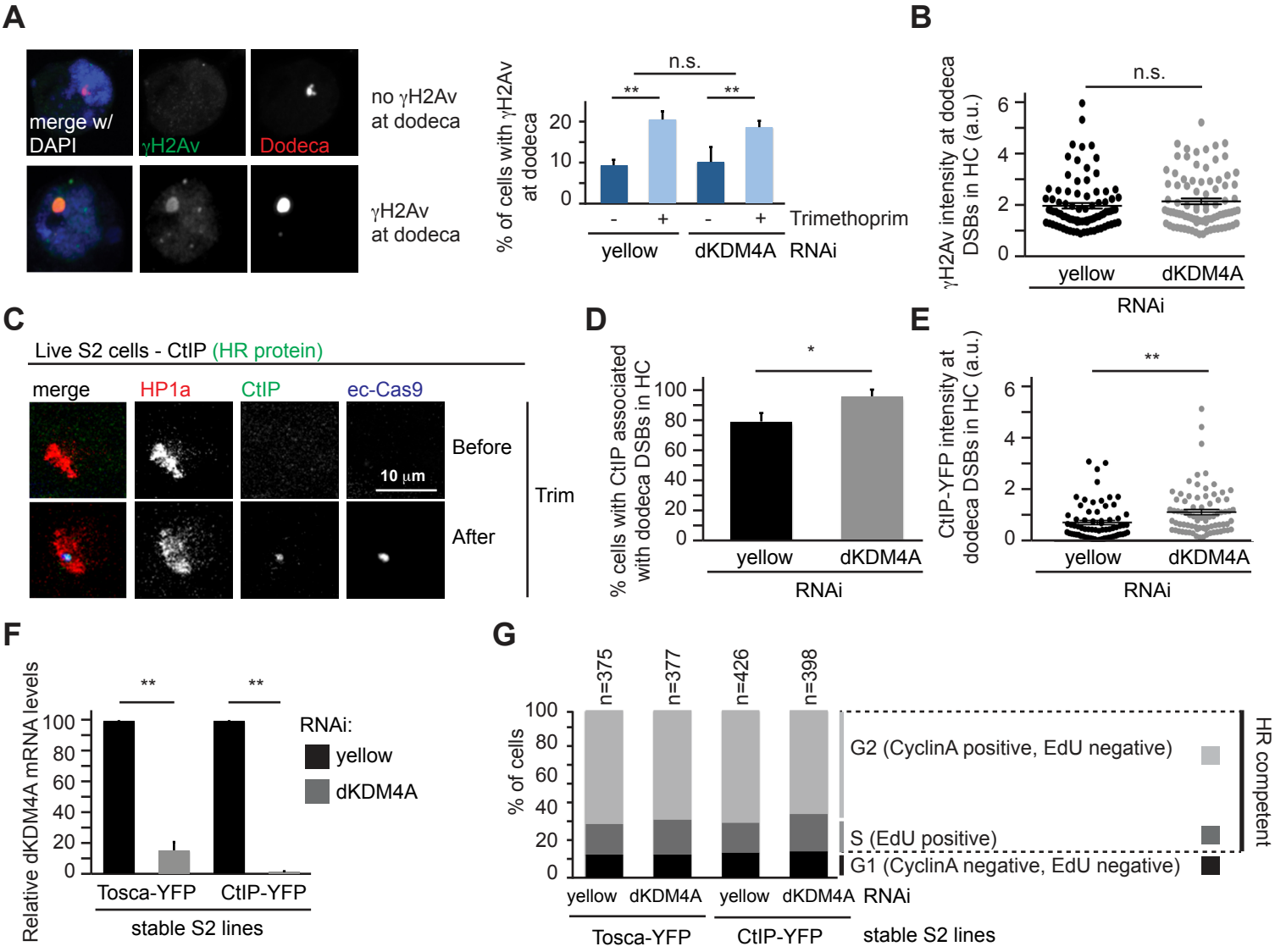
Supplemental figure 7



Supplemental figure 7.
A) Left: representative images of S2 cells immuno stained for γH2AV (green) in combination with FISH for the heterochromatic dodeca repeat (red). Right: Quantification of representative images (top) showing the number of S2 cells with γH2Av at dodeca repeats in heterochromatin. Cells expressing RNAi for yellow (control) or dKDM4A were transiently transfected with ecDHFR-Cas9 and dodeca gRNA and treated without (dark blue) or with (light blue) Trimethoprim to stabilize ecDHFR-Cas9. B) Quantification of the intensity of γH2Av foci at Cas9 induced DSBs at the dodeca repeat in the presence or absence of dKDM4A. C) Representative images of time-lapse movies of S2 cells stably expressing HP1a (red) and CtIP (HR protein, green) transiently transfected with fluorescently tagged inducible Cas9 (ecDHFR-Cas9, blue) and dodeca sgRNA. D) Quantification of the number of cells with CtIP protein localization to dodeca DSBs as performed in Fig.4B. E) Quantification of the level of CtIP present at Cas9 induced heterochromatic DSBs. Analysis was performed as in Fig. 4C. F) qRT-PCR analysis of dKDM4A mRNA levels in the presence of yellow (control) or dKDM4A dsRNA in indicated S2 cell lines. n.s. = p-value ≥ 0.05, * = p-value <0.05, ** = p-value <0.01 (t-test, unpaired). Averages + STDEV are plotted of n=3 experiments per condition. G) Cell cycle analysis of S2 cells transiently transfected with yellow (control) or dKDM4A dsRNA. Cells were fixed and stained on day 5 after transfection and cell cycle phase was determined based on EdU incorporation following 30 minutes of EdU exposure and immuno staining for Cyclin A; G2 (Cyclin A positive, EdU negative), S phase (EdU positive) and G1 phase (Cyclin A negative, EdU negative). Average percentages are shown with n= number of cells. H) Quantification of the number of cells with Rad51 foci in DAPI bright (heterochromatin) 60 minutes after 5Gy irradiation upon transfection with GFP RNAi (negative control, black), HP1a RNAi (positive control, red) and dKDM4A RNAi (grey). Cells were fixed 60 minutes after irradiation and immuno-stained for anti-Rad51 and DAPI (DNA). Averages are shown for one representative experiment + SEM. * = p-value <0.05 (t-test, unpaired). At least 38 cells were analyzed per condition.