



**Figure S6. Validation of hits with altered rDNA stability.** A) Dosage of cohesin subunits affects rDNA repeat loss rates. rDNA repeat loss rates in *RDN25-MAT $\alpha$*  strains overexpressing cohesin subunits *SMC1*, *SMC3*, *ECO1*, *SCC2*, *MCD1*, *RAD61* and *ESP1* are shown. Error bars represent standard deviation based on at least 4 biological replicates, as indicated by n. (B)-(D) H3K56 acetylation regulates rDNA stability and copy number. B) rDNA repeat loss rates in *rtt109 $\Delta$*  and *hst3 $\Delta$ hst4 $\Delta$*  mutants. C) rDNA repeat loss rates in various H3K56 point mutants. Error bars in (B) and (C) represent standard deviation based on 4 biological replicates. For (A)-(C) statistical significance was calculated using a standard 2-tailed t-test. \* -  $p < 0.05$ , \*\* -  $p < 0.01$ , \*\*\* -  $p < 0.001$ , \*\*\*\* -  $p < 0.0001$ , n. s. – not significant. D) Mutations that affect H3K56 acetylation are associated with expansion of the rDNA array. rDNA copy number was measured using ddPCR. Error bars represent standard deviation for each individual reaction.