



**Figure S4 Cas9 induced chromosomal translocations are not specific to the *ADE1* locus.** The same CRISPR-Cas9 mutagenesis protocol was performed but with the second gRNA targeting VPS35. Colonies in which cells had a fusion allele were identified by PCR as  $\Delta$ VPS35 cells have no easily identifiable phenotype. Breakpoint sequencing of 7 chromosomal translocations from three independent mutagenesis experiments confirmed the translocations. Fusion occurrences per replicate were 4 (4) /15, 3 (2) /16, and 1 (1) /16 (median rate: 18.75%). The number in parenthesis represents the number of detected breakpoints that were sequenced