

Figure S1

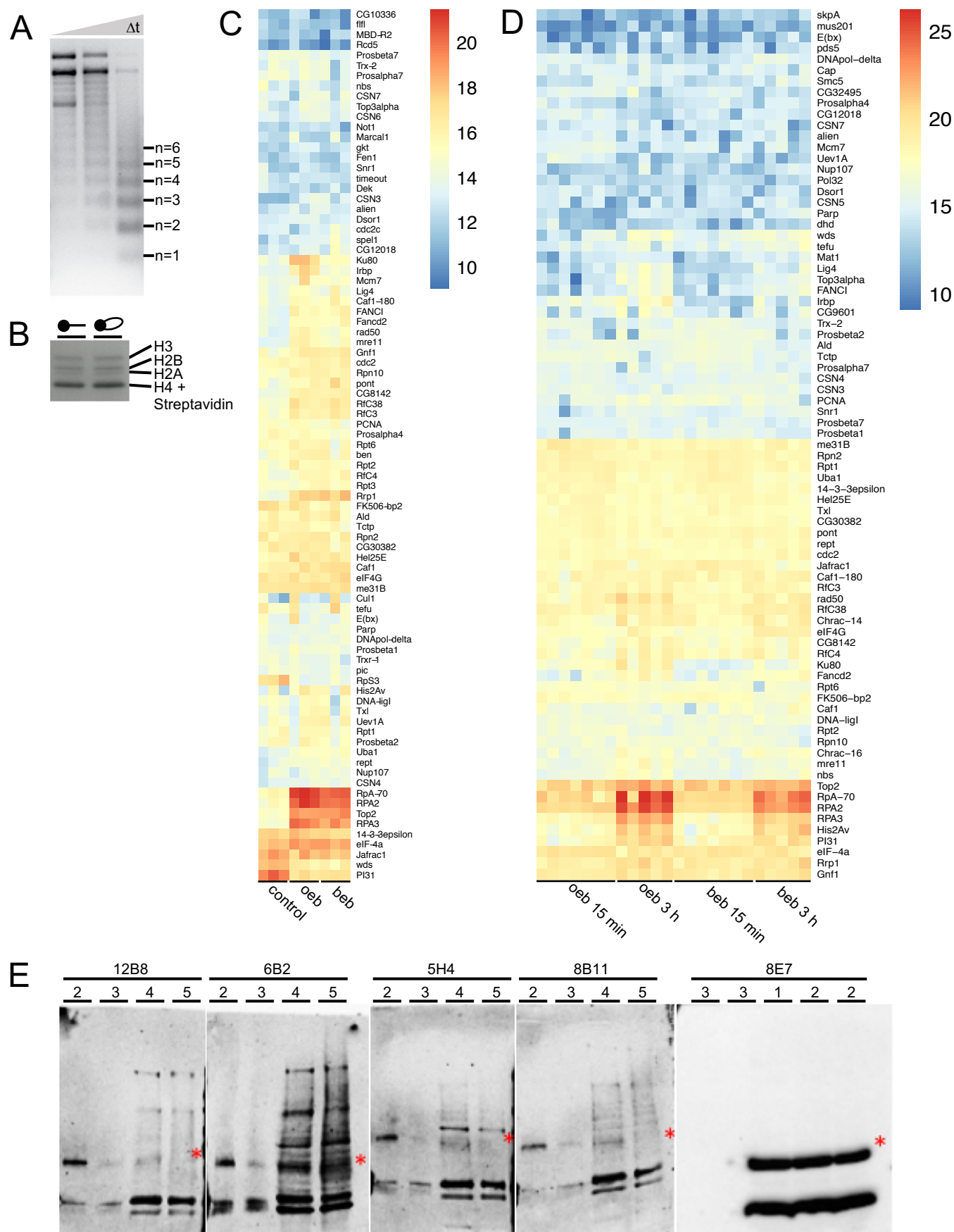


Figure S1:

- (A) MNase digestion of reconstituted chromatin for 15, 30 and 90 sec. n: the number of oligo-nucleosomes, with n=1: Mono-nucleosomes, n=2: di-nucleosomes, etc.
- (B) SDS gel electrophoresis and Coomassie staining of reconstituted chromatin on DNA immobilized at either one end or both ends after purification via paramagnetic beads.
- (C) Enrichment of factors associated with the GO term DNA damage in presence of no DNA (control), oeb or beb DNA from three biological replicates (see Figure 2B). High intensities are indicated in red, low intensities in blue. As control, beads only were incubated with extract.
- (D) Enrichment of factors associated with the GO term DNA damage in presence of oeb or beb DNA from seven biological replicates for 15 min assembly reactions and 5 biological replicates for 3 h assembly reactions (see Figure 2D). High intensities are indicated in red, low intensities in blue.
- (E) Western blot analysis to investigate the specificity and sensitivity of monoclonal antibodies against Ku70 and Ku80. M: Marker; 1: *Drosophila* embryo extract; 2, 3: *in vitro*-assembled chromatin on DNA immobilized either at one (2) or at both (3) ends; 4, 5: *Drosophila* S2 whole cell extracts from either wt (4) or RNAi knockdown (KD) cells (5). Membranes were probed with The Ku70 hybridoma were 12B8, 6B2, and 5H4 or Ku80 hybridoma were 8B11 and 8E7. Asterisks indicate bands of Ku70 and Ku80.

Figure S2

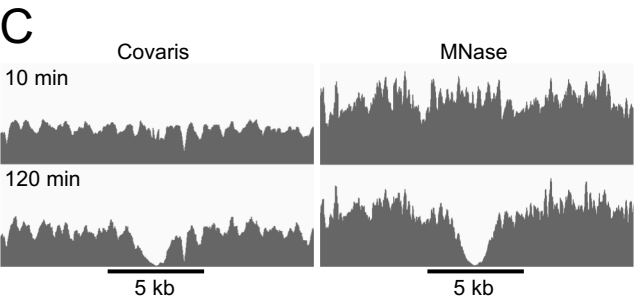
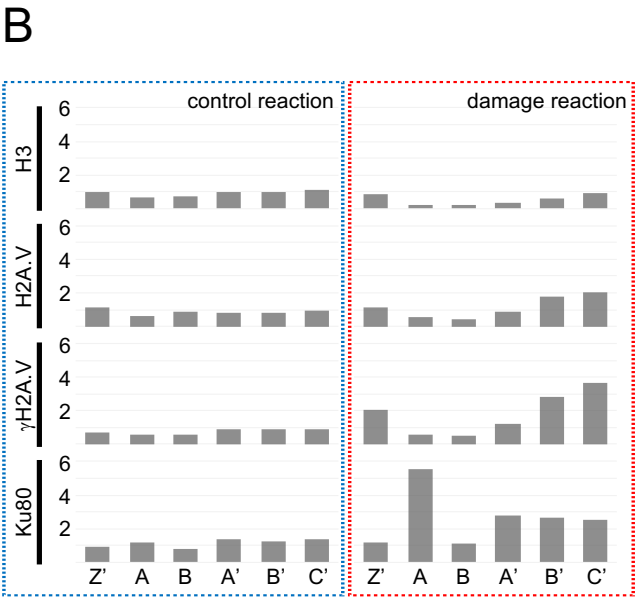
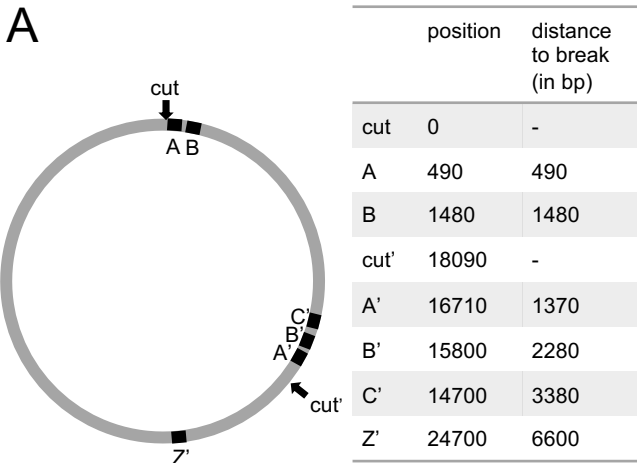


Figure S2:

(A) Map of linearized FlyFosmid with two cut sites (cut and cut') and qPCR target regions (A, B, A', B' and C'). Positions of cut sites and target regions and distances to the cut sites are listed in the table.

(B) ChIP-qPCR on chromatin assembled for 4 h on circular control FlyFosmids and on target FlyFosmids, which are either circular (control reaction) or linearized (damage reaction). Antibodies against H3, H2A.V, γH2A.V, and Ku80 were used. % of input was determined and normalized to the control FlyFosmid. The experiment was performed in one biological replicate.

(C) Resection of chromatin flanking free DNA ends analyzed by sequencing of MNase- or Covaris-sheared chromatin after 10 min or 120 min assembly.

Figure S3

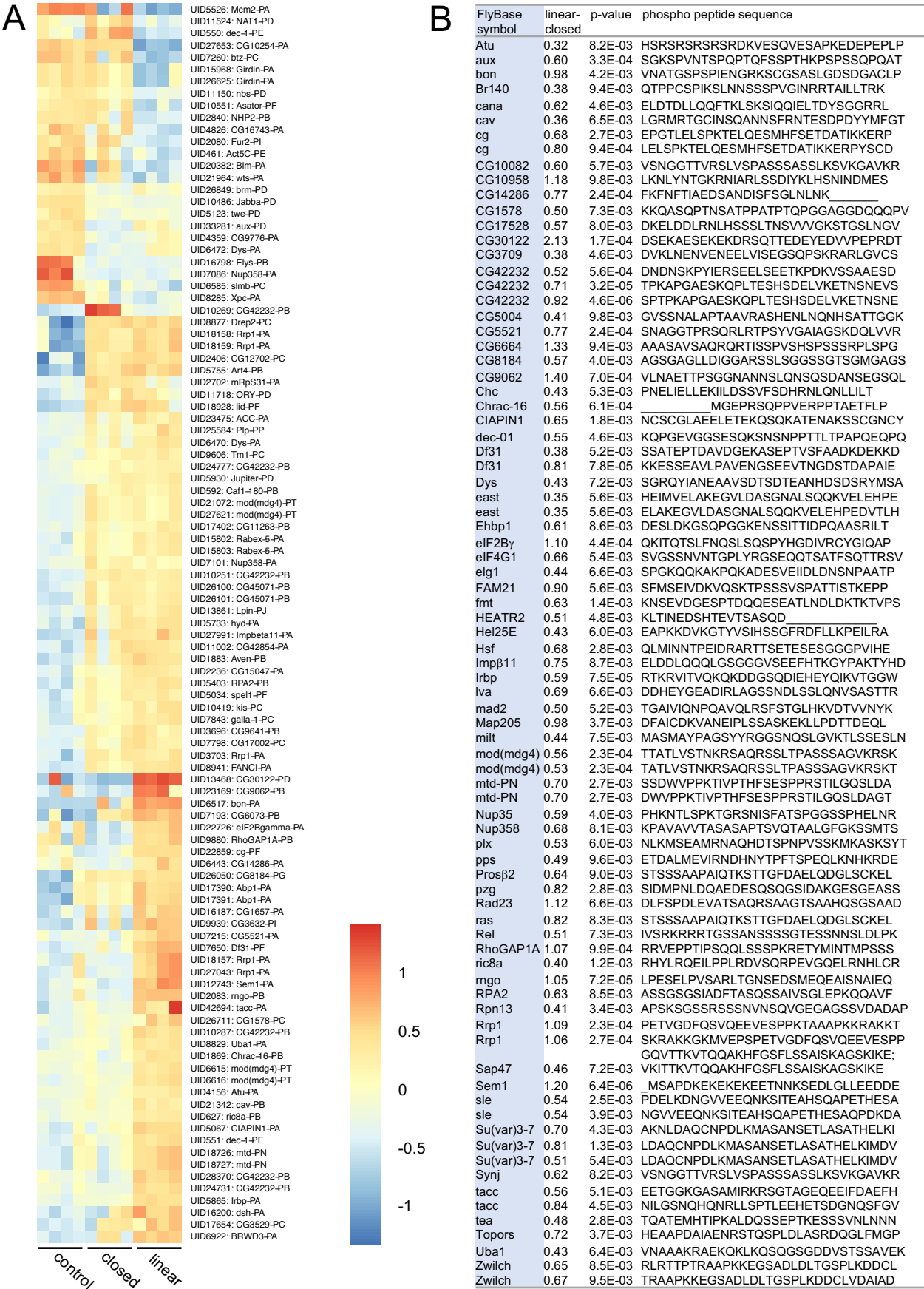


Figure S3:

- (A) Relative enrichment of phosphorylated peptides measured after assembly in absence of DNA (control), in presence of circular DNA (closed) or in presence of linearized DNA (linear) from four biological replicates . High intensities are indicated in red, low intensities in blue.
- (B) Proteins with phosphorylated peptides significantly increased in linear to circular DNA (see Figure 6B) are listed with their Flybase ID, their enrichment on linearized DNA in comparison to circular DNA, and their p-value. Proteins are sorted alphabetically. Each peptide sequence consists of a centred phosphorylated residue flanked by 15 amino acids. A full list of phosphopeptides can be found in Suppl. Table 3.

qPCR Primers Figure 5B and S2A, B

FlyFos019611

Z' fw: GGACCTGCTAGTGTCTCTGCG
Z' rv: GCAGATGGAACATTCCGTTCTGCG
A fw: GGCTGCGCCCTGTGC
A rv: GCTGTTCCCTGGTGCTTC
B fw: CCGGATGGCTCAGGCATCG
B rv: GCAGGAAGCGGCGGC
A' fw: CGGACGAGAAAGTGGTAAGAGGAGC
A' rv: CGACATAGAAACGTGTGCGTGCC
B' fw: CCAATGCACACACTCGAACTCACC
B' rv: CCACGAAGATGTCGGTAAACATTTGCG
C' fw: CCAGGGACCATCTCCACCTCC
C' rv: CGGCACACAAACTGTTTCGCC

FlyFos019829

fw: GGCCCGTTTGTCTAGAAAAAGAGCC
rv: GCCAGTCCAAGACGGTAACGC

FlyFos019611

1 fw: GGGGTCTCCCTTCAAGTAAGCC
1 rv: CGAGGTTGATTCAAGCTCCTCTTACC
2 fw: CGAGTGGCACAAGATTTATGCTCGG
2 rv: CGAAGCAGAAGAGCTTGGCACTG
3 fw: CGCATTGTTTCGCACAGACAAACTCG
3 rv: GCAGCTGCGTTTCAATCAGACC
4 fw: GGCTCACGTAGCTGAGTTTCTGG
4 rv: GCCTACGATTTCTATGGTCACGCC
5 fw: GTCTAAGCGCCGTGGGAATAGTTAACAC
5 rv: CACTCCTGATGGTACATGGCACC
6 fw: GCAGTGTGCCTAAATAAGCTCCGC
6 rv: GTTGTTGTCCTCGCAAAGCACTG

FlyFos019829

7 fw: GCACCCGTTTCGGAGTATCTGC
7 rv: GGGGCAGGAGCAGAGTATACC
8 fw: GCAGAGATTGGGAAGTGGTGGC
8 rv: CAGGGTGATGCCTGAGAGTCC