Figure S1

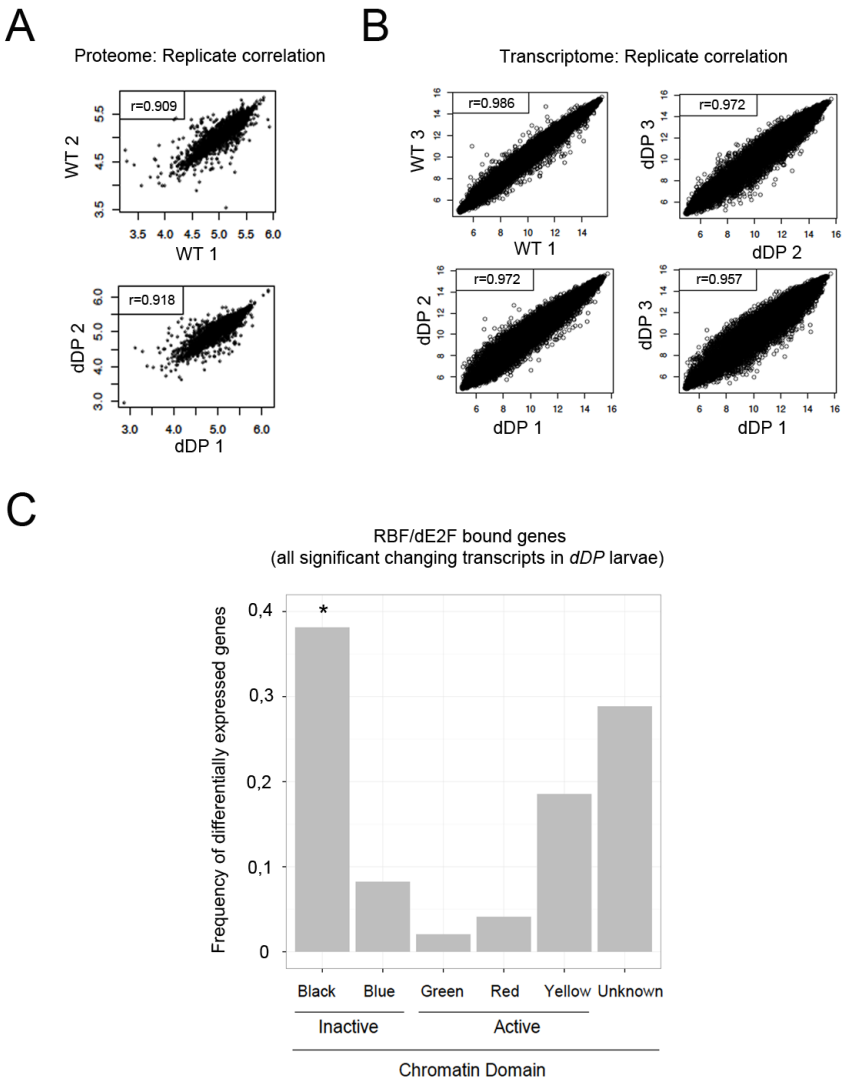

Figure S1, related to Figure 1. Biological correlation of datasets and chromatin loci distribution of differentially expressed transcripts. (A) Correlation plots between biological duplicates (proteome). (B) Correlation between transcriptome triplicates. (C) Frequency of chromatin localization of significant changing RBF/dE2F regulated loci to active/inactive domains (*p<0.05; Filion et al. 2010; data generated in Kc cells).

Figure S2

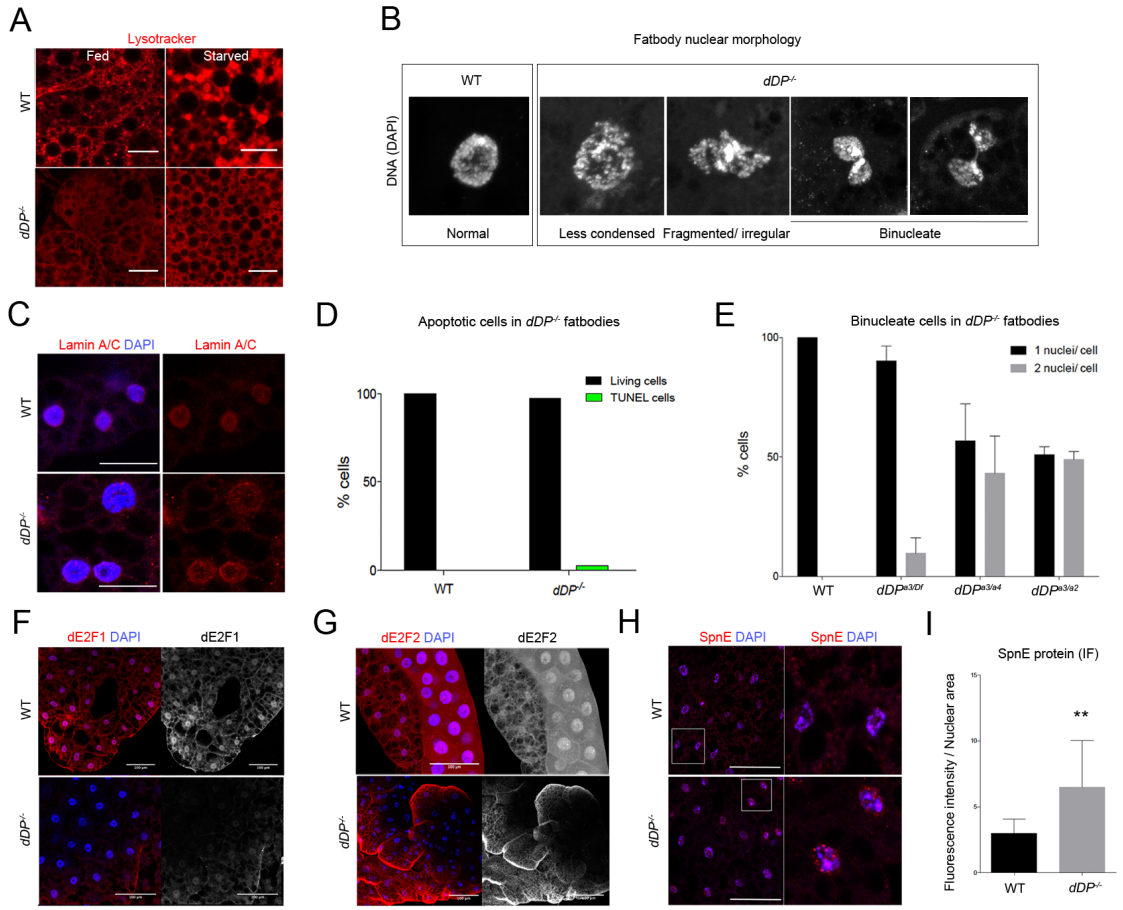

Figure S2, related to Figure 2. Loss of E2F activity in fat body cells. (A) Lysosomal vesicles in WT and *dDP*$^{-/-}$ fat bodies under normal and starvation conditions (scale bar, 20 μm). (B) Nuclear phenotypes in WT and *dDP*$^{-/-}$ fat bodies. (C) Lamin A/C is present in WT and *dDP*$^{-/-}$ fat body nuclei (scale bar, 30 μm). (D) TUNEL positive cells in WT (0%) and *dDP*$^{-/-}$ fat bodies (2.7%, n=37). (E) Mean ± SD % of binucleate cells in WT (n=60); *dDP*$^{a3/Df}$ (n=158); *dDP*$^{a3/a2}$ (n=110); *dDP*$^{a3/a4}$ (n=151). dE2F1 (F) and dE2F2 proteins (G) are absent in *dDP*$^{-/-}$ fat bodies (scale bar, 100 μm). (H) SpnE upregulation in *dDP*$^{-/-}$ FB cells (scale bar, 100 μm; mean ± SD of the intensity/nuclei area in I, unpaired t-test, **p<0.01).

Figure S3

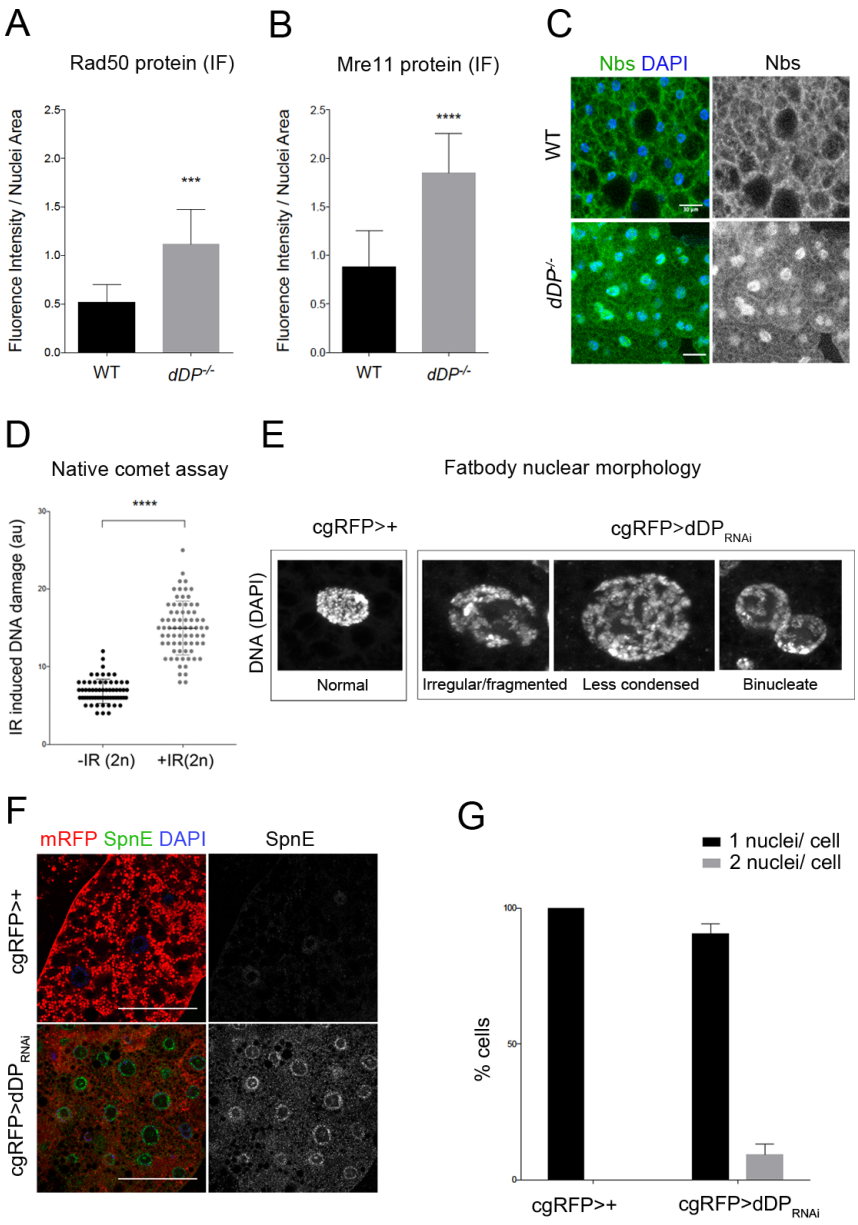

Figure S3, related to Figure 3. DNA Damage activity is increased in dDP depleted fat body cells. Rad50 (A) and Mre11(B) upregulation in *dDP*$^{-/-}$ fat body cells (mean ± SD of the intensity/nuclei area; unpaired t-test, ***p-val<0.001 and ****p-val<0.0001 respectively). (C) Nbs protein is upregulated in *dDP*$^{-/-}$ fat bodies (scale bar, 30 μm). (D) DNA damage induced in irradiated diploid cells (larval CNS, discs; IR, n=73; non-irradiated/-IR, n=62. Mann Whitney test, ****p-val<0.0001). (E) Nuclear morphology in cgRFP>+ and cgRFP>dDP$_{RNAi}$ fat bodies (F) Upregulated SpnE protein in cgRFP>dDP$_{RNAi}$ (control: cgRFP>+; scale bar, 100 μm). (G) Mean ± SD of the % of binucleated cells (cgRFP: n=300; cgRFP>dDP$_{RNAi}$:n=330).

Figure S4

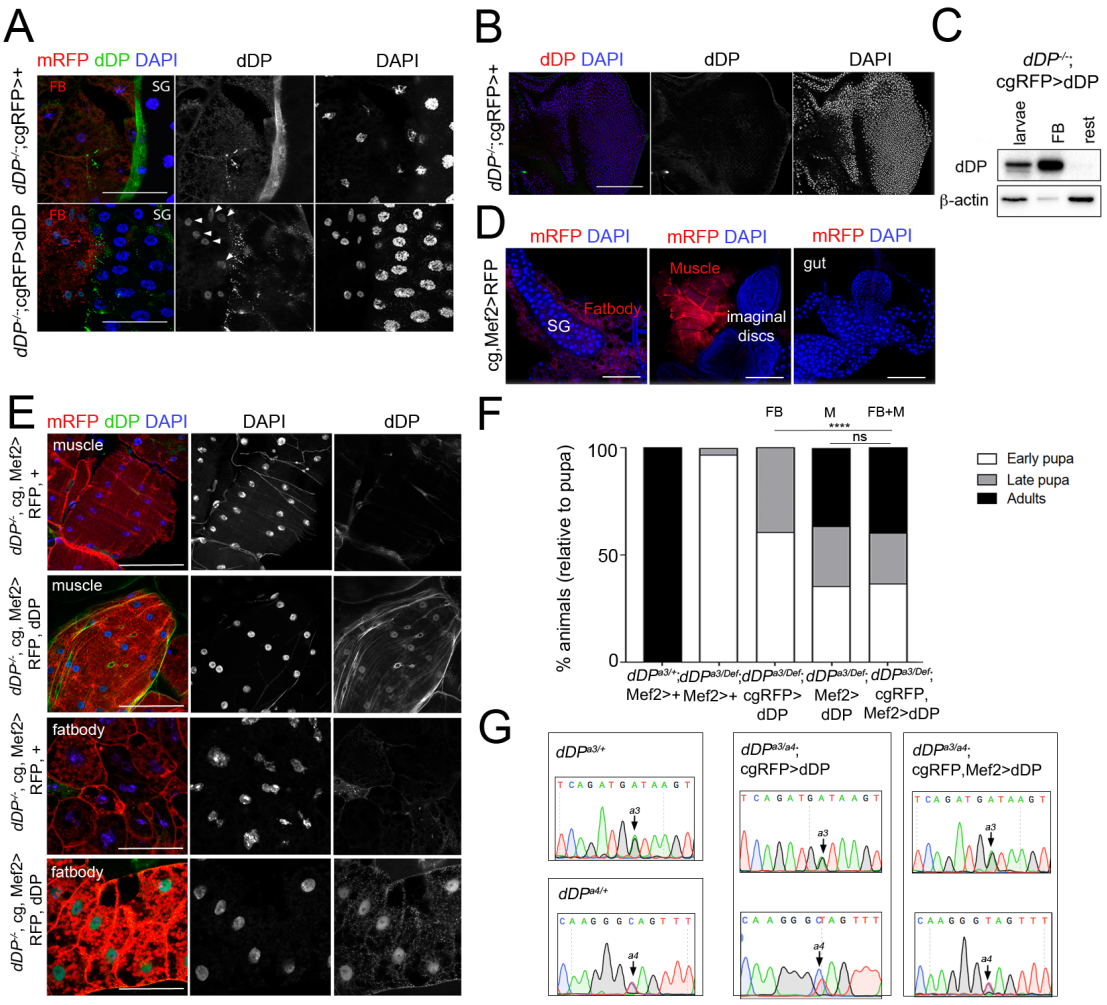

Figure S4, related to Figure 4. dDP re-expression in fat body cells rescues *dDP* defects. (A) dDP protein is specifically re-expressed in fat body cells of rescued animals (arrowheads; as control, adjacent salivary gland, SG; scale bars, 100 μm). (B) Absent dDP protein in *dDP*$^{-/-}$; cgRFP>+ eye discs; scale bars, 100 μm. (C) dDP blot of extracts from whole larvae, fat body and rest of larval tissues. (D) Combined expression of mRFP in muscles and fat body of cgRFP, Mef2>+ larvae (absent in other tissues; SG, discs and gut; scale bars, 200 μm). (E) dDP absence and re-expression in muscles and fat bodies of *dDP*$^{-/-}$; cgRFP, Mef2>+ and *dDP*$^{-/-}$; cgRFP, Mef2>dDP (scale bars, 100 μm). (F) % of animals arrested at each developmental stage: *dDP*$^{a3/+}$; Mef2>+are viable; *dDP*$^{a3/Df}$; cgRFP, Mef2>+ developed 0% adults and 3.22% pharates. *dDP*$^{a3/Df}$; cgRFP>dDP animals extended pupal development (39,32% pharates; n=178), and *dDP*$^{a3/Df}$; Mef2>dDP animals resulted in 27.7 % of pharates and 36.6% of adults (n=90). Combined re-expression of dDP in fat body and muscles in *dDP*$^{a3/Df}$; cgRFP, Mef2>dDP animals rescues more efficiently (n=30; 36.66% pupae, 23.33% pharates, 40% adults; Chi-squared test, ****p-val<0.0001 and ns, respectively). (G) Genotype of rescued animals and control mutant lines (*dDP*^{a3} and *dDP*^{a4} are point mutations described in Frolov et al., 2005).

Figure S5

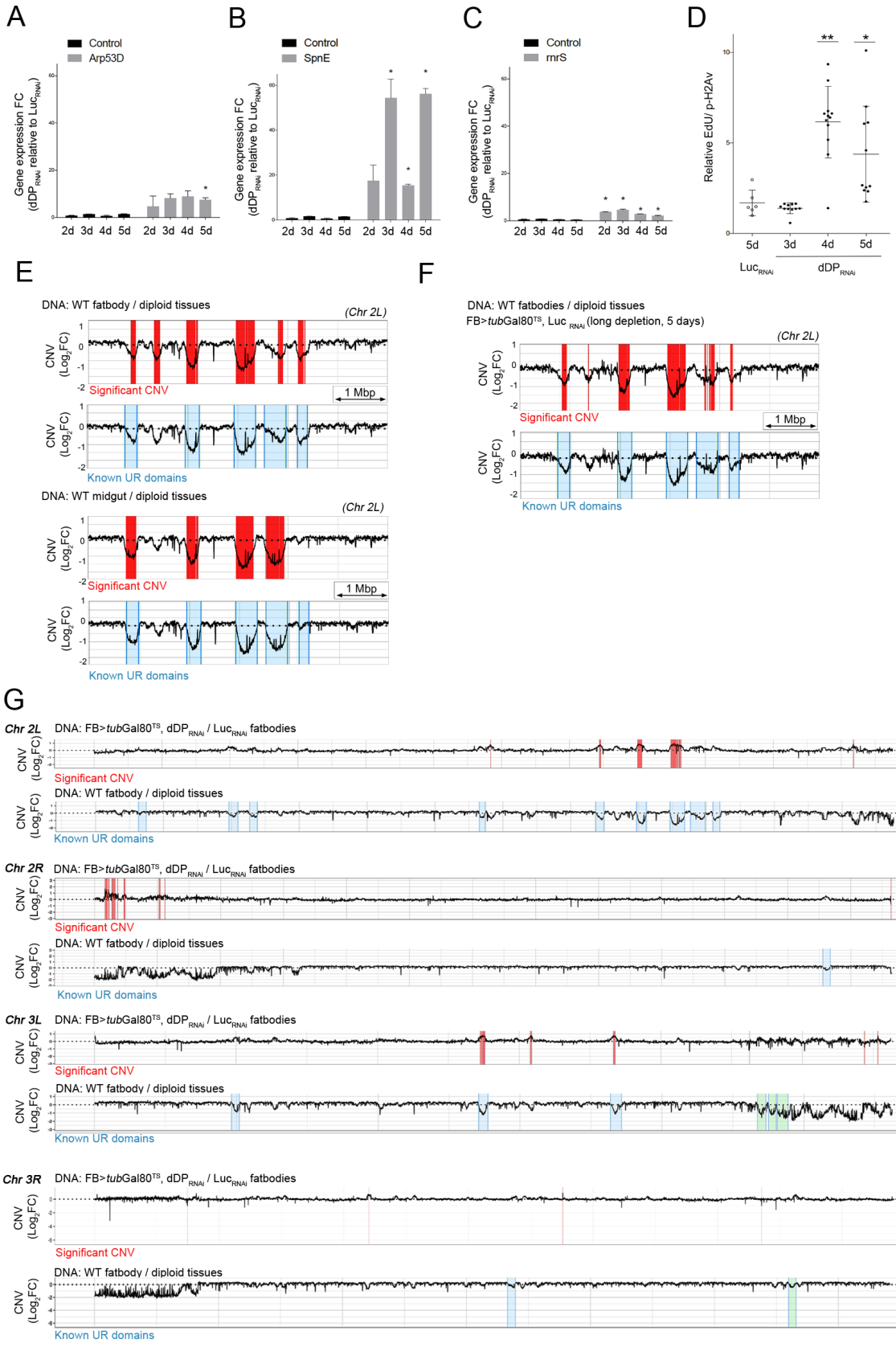
A
Control
Arp53D
Gene expression FC (dDP RNAi relative to Luc RNAi)
2d 3d 4d 5d 2d 3d 4d 5d
B
Control
SpnE
Gene expression FC (dDP RNAi relative to Luc RNAi)
2d 3d 4d 5d 2d 3d 4d 5d
C
Control
rnrS
Gene expression FC (dDP RNAi relative to Luc RNAi)
2d 3d 4d 5d 2d 3d 4d 5d
D
Relative EdU/ p-H2Av
5d 3d 4d 5d
Luc RNAi
dDP RNAi
E
DNA: WT fatbody / diploid tissues
(Chr 2L)
CNV (Log2FC)
Significant CNV
1 Mbp
Known UR domains
DNA: WT midgut / diploid tissues
(Chr 2L)
Significant CNV
1 Mbp
Known UR domains
F
DNA: WT fatbodies / diploid tissues
FB>tubGal80TS, Luc RNAi (long depletion, 5 days)
(Chr 2L)
Significant CNV
1 Mbp
Known UR domains
G
Chr 2L
DNA: FB>tubGal80TS, dDP RNAi / Luc RNAi fatbodies
Significant CNV
DNA: WT fatbody / diploid tissues
Known UR domains
Chr 2R
DNA: FB>tubGal80TS, dDP RNAi / Luc RNAi fatbodies
Significant CNV
DNA: WT fatbody / diploid tissues
Known UR domains
Chr 3L
DNA: FB>tubGal80TS, dDP RNAi / Luc RNAi fatbodies
Significant CNV
DNA: WT fatbody / diploid tissues
Known UR domains
Chr 3R
DNA: FB>tubGal80TS, dDP RNAi / Luc RNAi fatbodies
Significant CNV
DNA: WT fatbody / diploid tissues
Known UR domains

Figure S5, related to Figure 5. Time controlled depletion of dDP in the fat body results in DNA replication. (A-C) Mean ± SD gene expression fold changes of de-repressed dE2F target genes (Arp53D, SpnE and rnrS) in FB>*tub*G80^{TS}>GFP, UAS>dDP$_{RNAi}$ fat body RNA (relative to UAS Luc$_{RNAi}$; ANOVA, *p-val<0.05). In black, the mean ± SD of Gapdh and Rpl32 as control. (D) Relative EdU/p-H2Av signal quantification (n=10-12 nuclei/ time-point; Kruskal-Wallis, **p<0.01 and *p<0.05 respectively). (E) Inset of chromosome 2L: significant CNV (red) in WT fat body and midgut, relative to diploid tissues (known under-replicated (UR) domains, in blue). (F) Control depletion in FB>*tub*G80^{TS}>GFP,UAS Luc$_{RNAi}$ fat bodies do not show replicating regions (significant CNV, in red) in known under-replicated regions (UR, blue). Genomic scale: 1 Million bp (Mbp). (G) significant replicating regions (in red) upon 5 days of dDP depletion in 2R, 2L and 3R, 3L chromosomes (CNV calculated between DNA of FB>*tub*G80^{TS}>GFP, UAS dDP$_{RNAi}$ and UAS Luc$_{RNAi}$ in known under-replicated regions; $Log_2FC>0.6$; FDR<0.001).

Figure S6

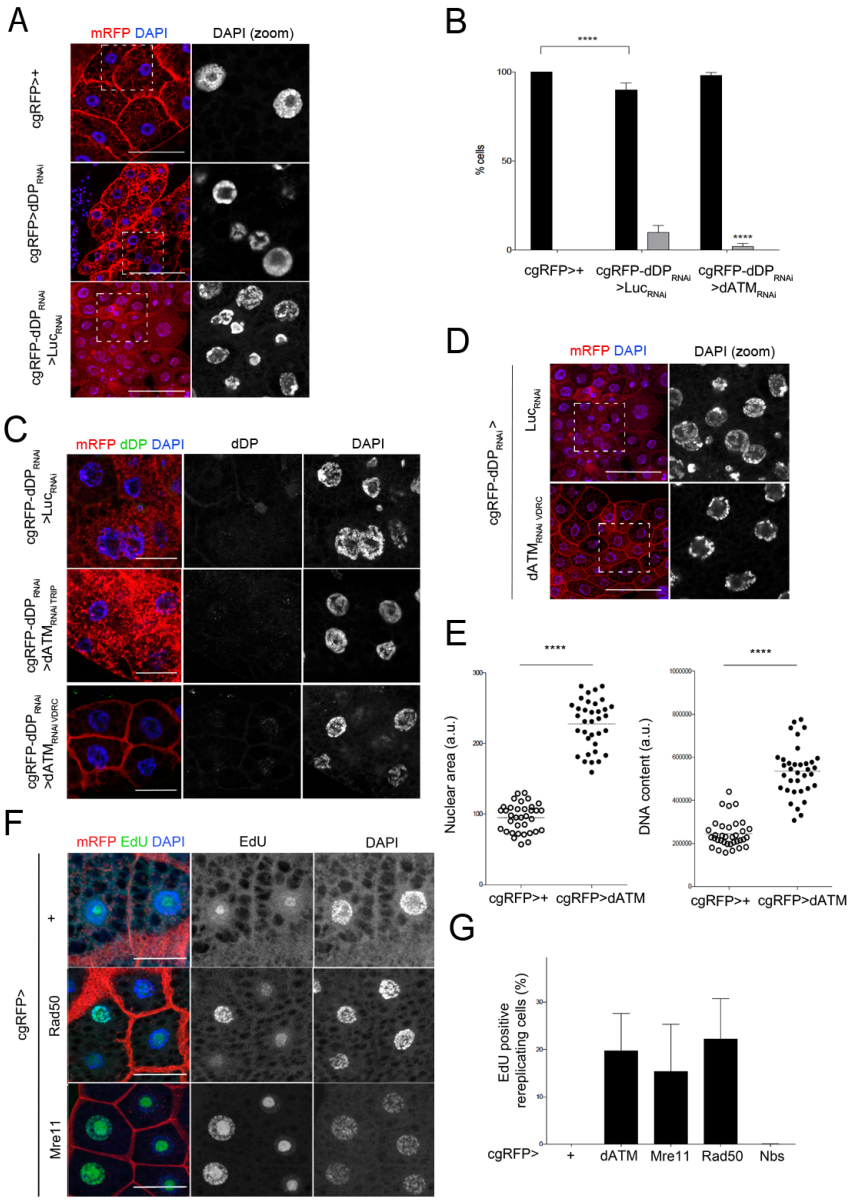

Figure S6, related to Figure 6. dATM activity is critical in dDP depleted fat body cells. In all cells, DNA is labeled with DAPI and mRFP is tagged to the membrane. (A) Nuclear defects of cgRFP>dDP$_{RNAi}$ (middle) are similar to the of cgRFP-dDP$_{RNAi}$>Luc$_{RNAi}$ nuclei defects (control, cgRFP>+; scale bar, 100 μm). (B) Mean ± SD % of binucleate cells in cgRFP, cgRFP-dDP$_{RNAi}$>Luc$_{RNAi}$, cgRFP-dDP$_{RNAi}$>dATM$_{RNAi}$ fat bodies (n= 100, 206, 262 cells respectively; ANOVA, ****pval<0.0001). (C) dDP protein is absent in cgRFP-dDP$_{RNAi}$>Luc$_{RNAi}$ and cgRFP-dDP$_{RNAi}$>dATM$_{RNAi}$ fat bodies (scale bar: 40 μm). (D) An alternative UAS dATM $_{RNAi\ (VDRC)}$ line rescues nuclear defects (compare with cgRFP-dDP$_{RNAi}$>Luc$_{RNAi}$; scale bar, 100 μm). (E) Individual plot measurements showing an increase in nuclear area (fold change= 2.42) and in DNA content (fold change= 2.01) of cgRFP>dATM cells (compared to control, cgRFP>+ nuclei, n=35; Mann-Whitney test, ****p<0.0001; au=airy units). (F) EdU in cgRFP>dRad50, Mre11 fat bodies (control, cgRFP>+; scale bar, 40 μm). (G) Mean ± SD of % EdU positive cells: cgRFP>dATM (19.74%), >Mre11 (15.41%), >Rad50 (22.21%), >Nbs (0.05%) (n=239, 260, 270, 500 cells, respectively).

Figure S7

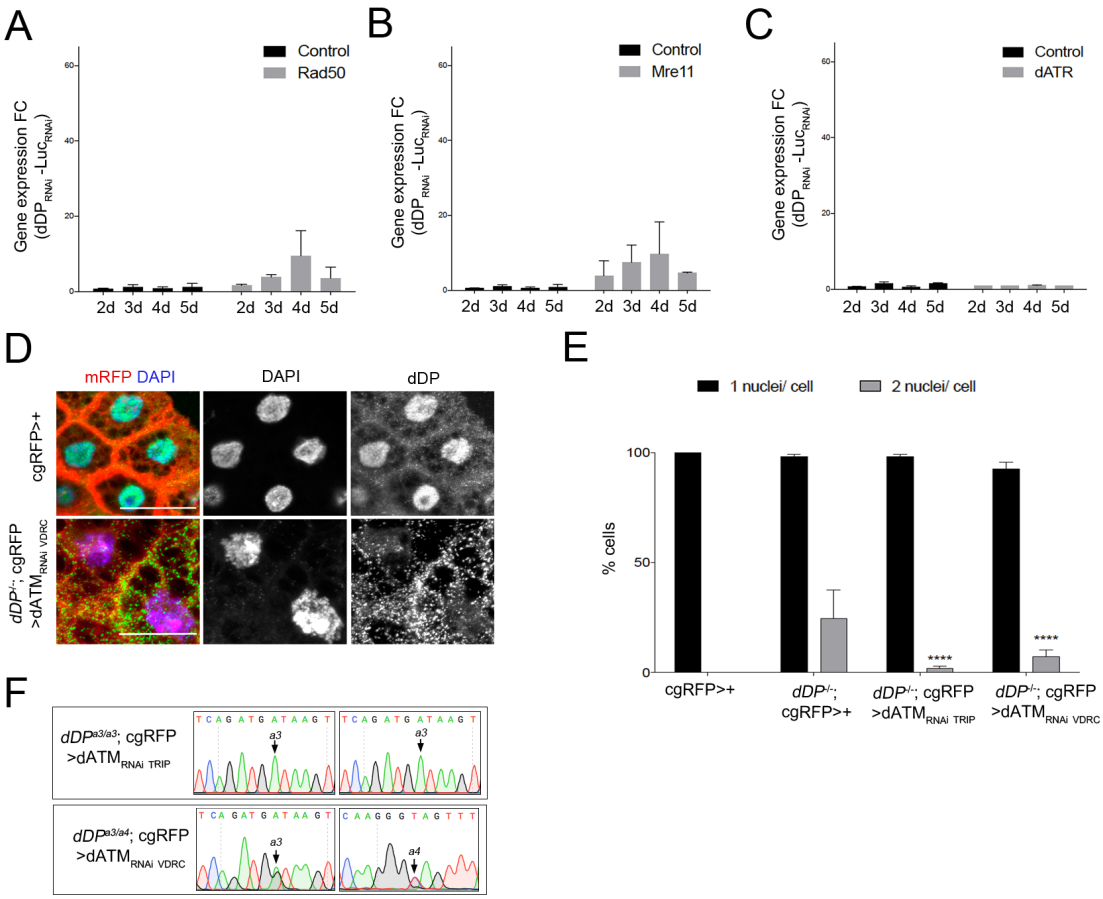

Figure S7, related to Figure 7. Elimination of dATM rescues *dDP* phenotypes. (A-C) Fold change in expression of Rad50 (A), Mre11 (B) and ATR (C) in FB>*tub*G80^{TS}>GFP, UAS>dDP$_{RNAi}$ fat body RNA (compared to UAS Luc$_{RNAi}$; Gapdh and Rpl32 are used as control transcripts; ANOVA, ns). (D) Rescue of nuclear defects in the fat body of *dDP$^{-/-}$;* cgRFP>dATM $_{RNAi\ (VDRC)}$ animals; scale bar, 25 μm. (E) Significant reduction of the mean ± SD of binucleate cells in both *dDP$^{-/-}$;* cgRFP>dATM $_{RNAi}$ larvae (n= 100,147,267 and 199, respectively; ANOVA, ****pval<0.0001). (F) Genotype of *dDP$^{-/-}$;* cgRFP>dATM $_{RNAi}$ animals (*dDP^{a3}* and *dDP^{a4}* are point mutations described in Frolov et al., 2005).

Table S4

qPCR oligonucleotides
tefu(F): GGGATTCGATAAACTGGC
tefu(R): AAAGGCAGCAGGCAGGTCTT
ATR(F): CCCTCTCTGGGAAGAATCGTG
ATR(R): CTTAACGCTCTCGTTGTC
Rad50(F): CGGAGTTTCGGCACCTATG
Rad50(R): TCTTTCCGCATCCGTTCTCG
Mre11(F): AACCAGTCGGTGAATTACGAGG
Mre11(R): CGTCGTGATTGCCATGAATAGAG
SpnE(F): TGATCGGCACCGACTATGTCA
SpnE(R): CTTGGCGTAGATGGACAAGTT
rnrS(F): CGTCCAAGGAAAACATTGCTG
rnrS(R): TGGTGCTATCCGTCAGAATCTT
Rpl32(F): AGCATACAGGCCCAAGATCG
Rpl32(R): TGTTGTCGATACCCTTGGGC
Gapdh(F): TAAATTCGACTCGACTCACGGT
Gapdh(R): CTCCACCACATACTCGGCTC
Genotyping primers
Genotype (F): CCAGAACAAGTCCGAAATGG
Genotype (R): TGGTAAGAGGAGGATCACACG

Table S4, related to STAR Methods. All qPCR oligonucleotides are designed with FlyPrimerBank (Hu et al. 2013). Genotyping primers are described in Zappia and Frolov, 2016.