Valadez_Sup. Table 1 T1.

Supplementary information.

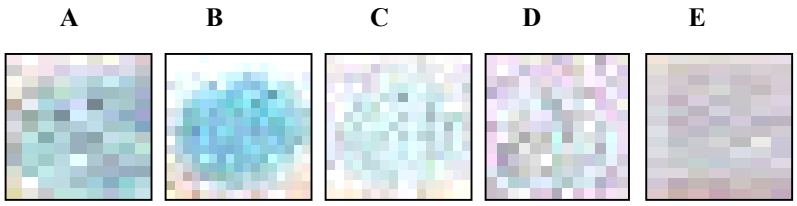
Supp. Table 1. Clones identified by a yeast-hybrid screening with XNP/dATRX.

CLONE	HIT IN FLYBASE
A 1-3 40 ab1	CG4440 putative DEAD-box helicase
40 ab2	CG10597 RNA helicase
47 cb-1	CG33526 PNUTS
10ab1	CG3048 TRAF1
63 cb1	CG5163 TFIIA-S
A14	CG 3333 NOP60B
1 ab2 1 ab4	CG5838 DREF
21 ab2	CG6341 elongation factor beta 1

CLONE	HIT flybase
17 cb2	CG12750 nucampholin
26 cb1	CG2238 EF2b
51 cb1	CG11051 NPLP2
51 cb2	CG15213
C 45	CG42281 BUNCHED
25 cb2	CG17521 Qm
55 cb2	CG5920 SOP
47 cb3	CG9922
51 cb2	CG9034
58 cb2	CG9995 HTT
C64 cb97	CG16738 Slp1

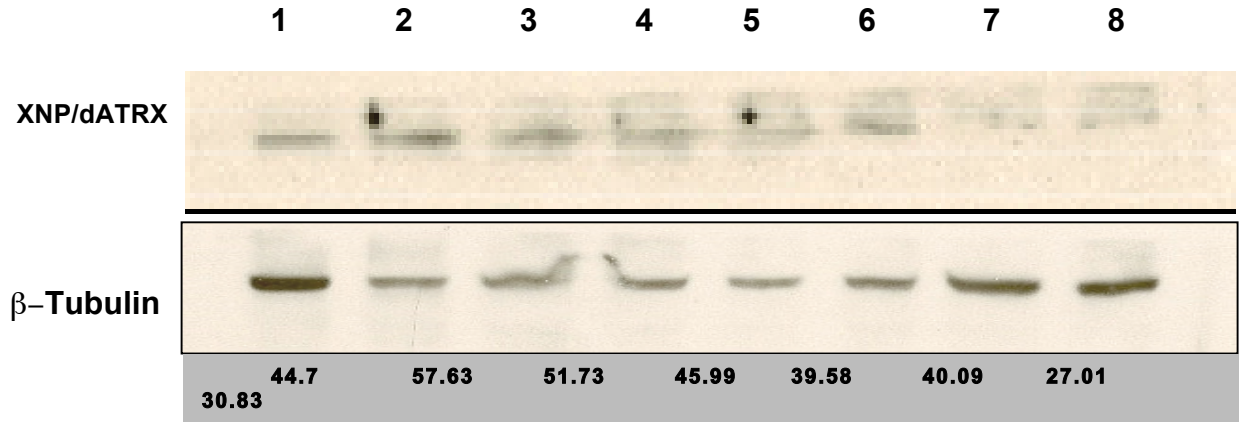
Supp. Table 1. **XNP/dATRX-interacting clones identified by the yeast two-hybrid screening**. Clone number indicates interaction with dATRX N-terminus (ab) or C-terminus (cb). Note that several clones encode transcription factors or proteins that participate in chromatin dynamics.

Valadez_Supp. Fig. 1 S1

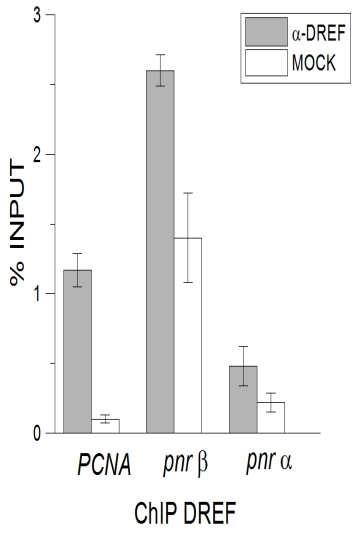


Supp. Fig. 1. Growth in selective QDO media and β-galactosidase activity of yeast expressing the N-terminal domain of XNP/dATRX and DREF. A) Positive control of the interaction between SV40 antigen with p53. B) Interaction between the N-terminal region of XNP/dATRX with the CR3 domain of DREF. C) Interaction between the C-terminal region of XNP/dATRX and CG9922. D) Negative control, SV40 antigen-lam. E) Negative control, pGBKT7-DREF.

Valadez_ Supp. Fig 2 S2.

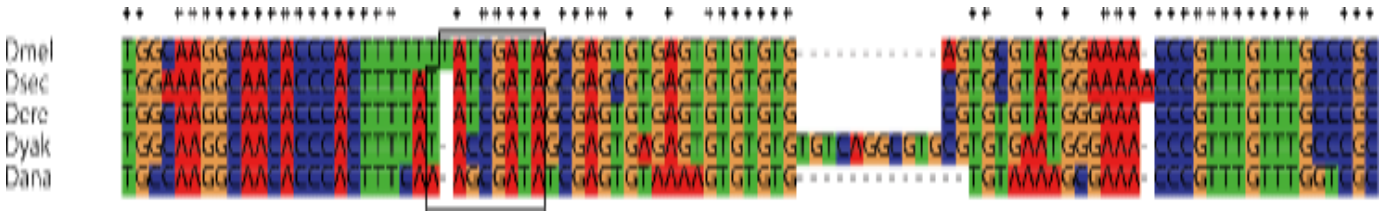


Supp. Fig 2. XNP/dATRX levels in protein extracts from adult flies thoraxes with different XNP/dATRX and *pnr* genotypes. In the upper panel the Western blot was performed against XNP/dATRX and in the lower panel an anti β-tubulin antibody was used as a loading control. The genotypes in each protein preparation are: lane 1 *OreR;* Lane 2, *UAS-dATRXi/UAS-dATRXi;* Lane 3, *pnr-Gal4/+*; Lane 4, *UAS-dATRXi/+*; *pnr-Gal4/+*; Lane 5, *UAS-dATRXi/UAS-dATRXi ; pnr-Gal4/+*; Lane 6 *UAS-dATRXi/+ ; pnr-Gal4/pnr-Gal4*; Lane 7 and 8 *UAS-dATRXi/UAS-dATRXi*; *pnr-Gal4/pnr-Gal4*. XNP/dATRX levels are lower in flies with two copies of the inducible RNAi than in flies with one copy. Numbers at the bottom are the relative value between XNP/dATRX and β-tubulin after densitometric analysis of the bands.



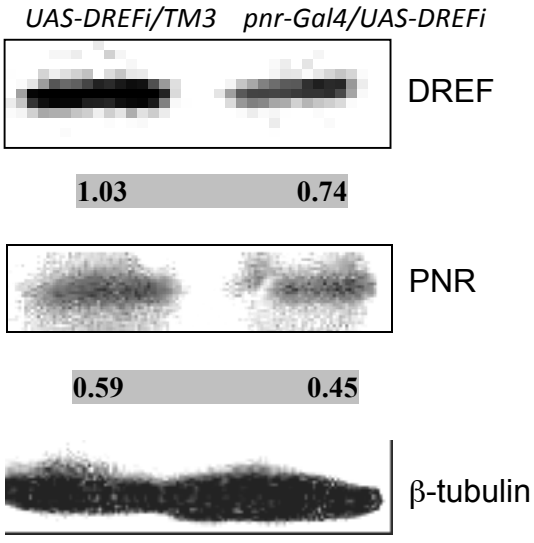
Supp. Fig 3. **ChIP assay using a specific antibody against DREF to determine if the DREF factor is poised in the *DRE-pnrα* and *DRE-pnrβ* elements in chromatin derived from wild type embryos**. As a positive control we used a PCNA sequence that is recognized by DREF. Graphs show the results from three independent immunoprecipitation reactions (n=3). ChIP signals quantified by means of quantitative polymerase chain reaction, are presented as % of the input. As it can be observed in the figure, the *PCNA* and *pnrβ* chromatin regions are significantly enriched when the precipitation was carried out with the DREF antibody. The *DRE-pnrα* region does not show a significant difference with the control.

Valadez. Supp. Fig.4 S4



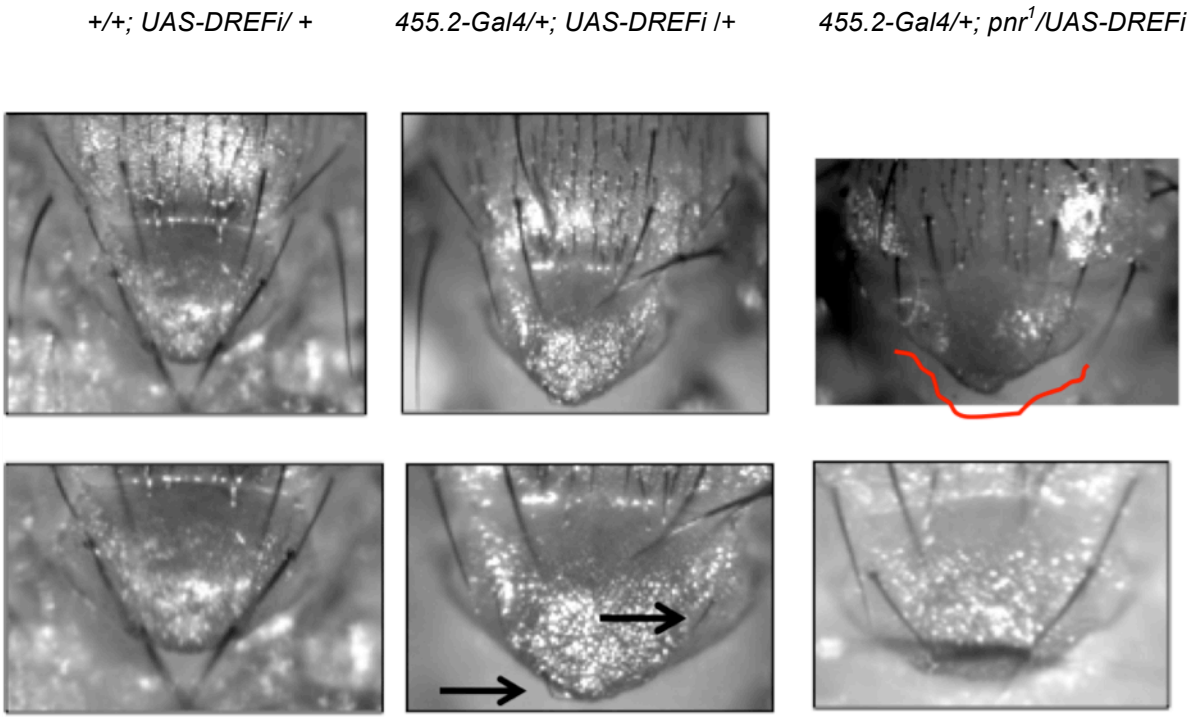
Supp. Fig 4. A genomic region upstream of *pnr-β* is highly conserved among the *melanogaster* group. The genomic regions located upstream of the *pannier* transcription unit in *Drosophila melanogaster* were compared among available sequences of *Drosophila* species using Flybase Blast (http://flybase.net/blast/). After identifying a conserved region in some of these *Drosophila* species, the corresponding sequences were aligned using the Clustal X program. In this region the putative DNA replication element (DRE, indicated by the box) is identical in *D.melanogaster, D. sechellia and D.erecta.* Compared to *D. melanogaster* sequences *D. yakuba* has one mismatch, *D. ananassae* has two base pair differences, nevertheless all of them conserve the GATA motif at the end of the octamer. Note that nucleotide sequence around the DRE element is highly conserved among the *melanogaster* group, suggesting that this region has a relevant role in the regulation of *pnr.*

Valadez_Supp. Fig. 5 S5



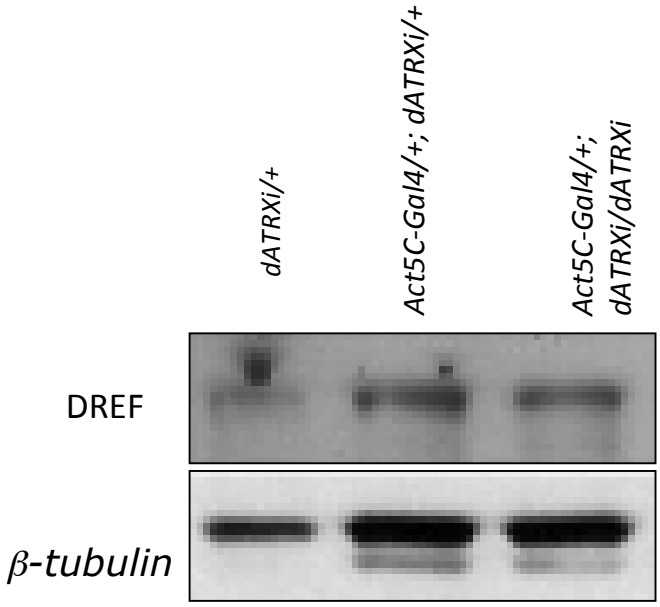
Supp. Fig. 5. Reduction of DREF and PNR protein levels in the thorax of DREF knockdown flies. Specific antibodies were used to detect DREF (upper panel) and PNR (middle panel). An anti β-tubulin antibody was used as a loading control (bottom panel). Genotypes of the flies are indicated. Numbers represent the value of the relation between DREF and Pnr with β-tubulin after densitometric analysis of the bands.

Valadez_Supp. Fig. 6 S6.



Supp. Fig. 6. Depletion of DREF in the scutellum reduces or eliminates the machrochaetes and in combination with *pannier* alleles the scutellum is deformed. The genotypes are indicated in the figure. Arrows show the reduction or absence of bristles. Scutellum size is smaller with irregular borders (shown by the red line) in *455.2-Gal4/+*; *pnr*^{1}*/UAS-DREFi* flies.

Valadez- Supp. Fig 7



Supp. Fig. 7. Depletion of XNP/dATRX by RNAi in adult organisms does not affect DREF levels. DREF levels were detected in adult flies protein extracts expressing an RNAi against XNP/dATRX using the *Act5C-Gal4* driver or without it. Tubulin was used as a loading control. Genotypes are indicated in the figure.