

Figure S8. Characterization of DREF depletion

- **A.** Graph representing the results from 3 independent RT-qPCR analyses to measure the mRNA levels of *actin*, *beaf32*, *dMes-4*, and *dref* upon knock-down of Beaf32 ('Beaf32-KD'; green bars), dMes-4 ('dMes-4-KD; red bars), DREF ('DREF-KD'; purple bars) or control ('control'; black bars). The error bar corresponds to the standard deviation of the measure. The y axis shows the relative fold change in expression normalized to *actin* control.
- **B.** Western blotting analysis of nuclear extracts using anti-DREF antibodies. The arrow indicates the unique band detected corresponding to the molecular weight of DREF (713 AA).
- C. Western blotting analysis of nuclear extracts prepared from DREF-KD or control cells using anti-DREF or anti-actin control antibodies for loading control. The efficiency of DREF-KD was confirmed by RTqPCR analysis (see panel A).
- **D.** Quantification of DREF depletion as compared to loading control from the western blotting analysis in panel C (see Methods).
- **E.** Box plot showing the results of ChIP performed in DREF-KD (red boxes) compared to WT-control (mock-depleted) cells (green boxes) in percent of input (y-axis) with anti-dMes-4 antibodies or IgG control, for 16 promoters harboring a Beaf32 site or not, as indicated (see Methods for a list of genes). ChIP data were analyzed by qPCR analyses in triplicates and for three independent measures normalized to three control loci (see Methods).
- **F.** Co-immunoprecipitation experiment using anti-DREF antibodies performed in parallel with IgG control followed by western blotting analysis using anti- Beaf32, DREF or -histone H3 as loading control, compared to input nuclear extract (10% 'input').