Supplementary Figures

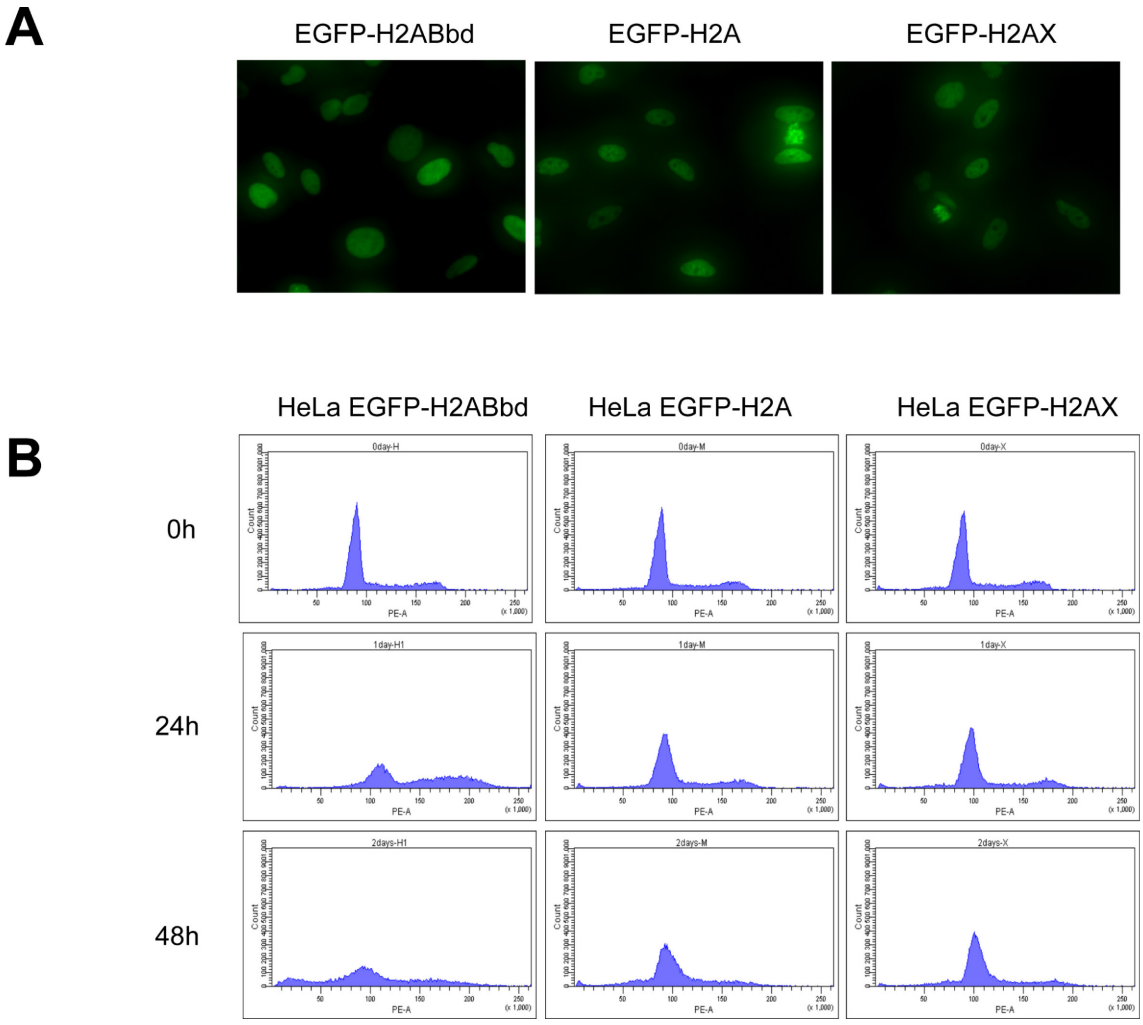

Figure S1.

Expression and effect of the H2ABbd on cell growth.

(A) The efficiency of transduction was monitored by microscopy. HeLa cells infected with lentivirus expressing the EGFP-tagged H2ABbd, H2A or H2AX were treated with Dox for 24 hr. Representative pictures are shown. **(B)** At the indicated time points after induction of EGFP-H2AX, HeLa cells were fixed with ethanol and FACS analysis was performed. Expression of H2ABbd, but not H2A or H2AX, caused abnormal cell cycle pattern and increased sub G1 cells.

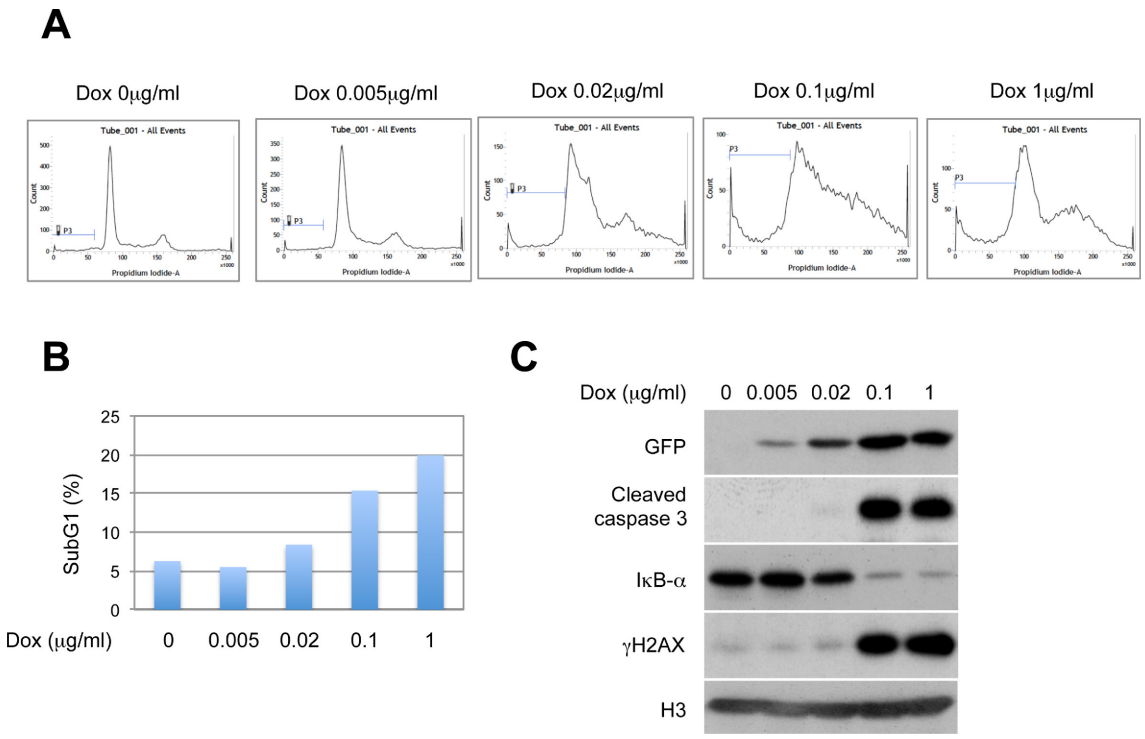

Figure S2.

Low and moderate expression of H2ABbd did not trigger apoptosis

(A) HeLa cells transfected with Dox inducible EGFP-H2ABbd were cultured in the presence of Dox (0μg/ml, 0.005μg/ml, 0.02μg/ml, 0.1μg/ml, 1μg/ml) for 48h. Samples were collected and fixed with ethanol and FACS analysis was performed. Area described as P3 means sub G1 cells. **(B)** Graph represents percentage of sub G1 cells. **(C)**. Immunoblot analysis was performed using the indicated antibodies.

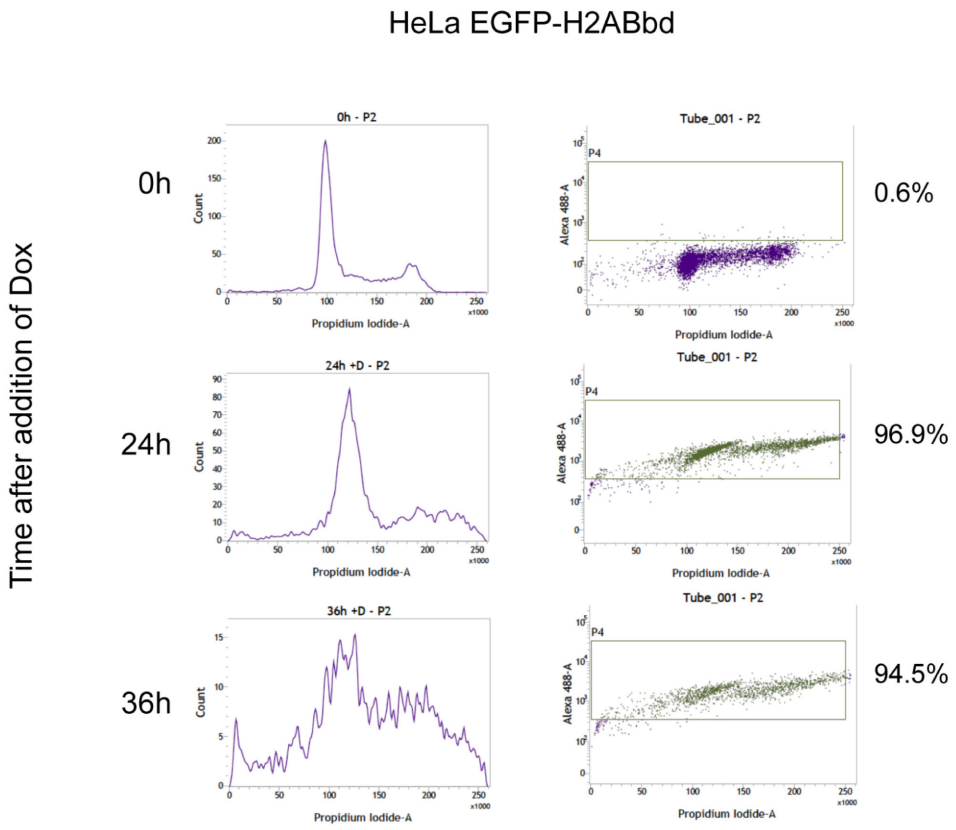

Figure S3.

γH2AX positive cells were detected in S phase

HeLa cells transfected with Dox inducible EGFP-H2ABbd were cultured in the presence of Dox. Cells were collected at the indicated time points, fixed with ethanol and double stained with propidium iodide (PI) and γH2AX-Alexa 488. The left panels show PI histograms and right panels show a double stained analysis. Cells were sorted for γH2AX incorporation and DNA content. The percentages of γH2AX positive cells are indicated.

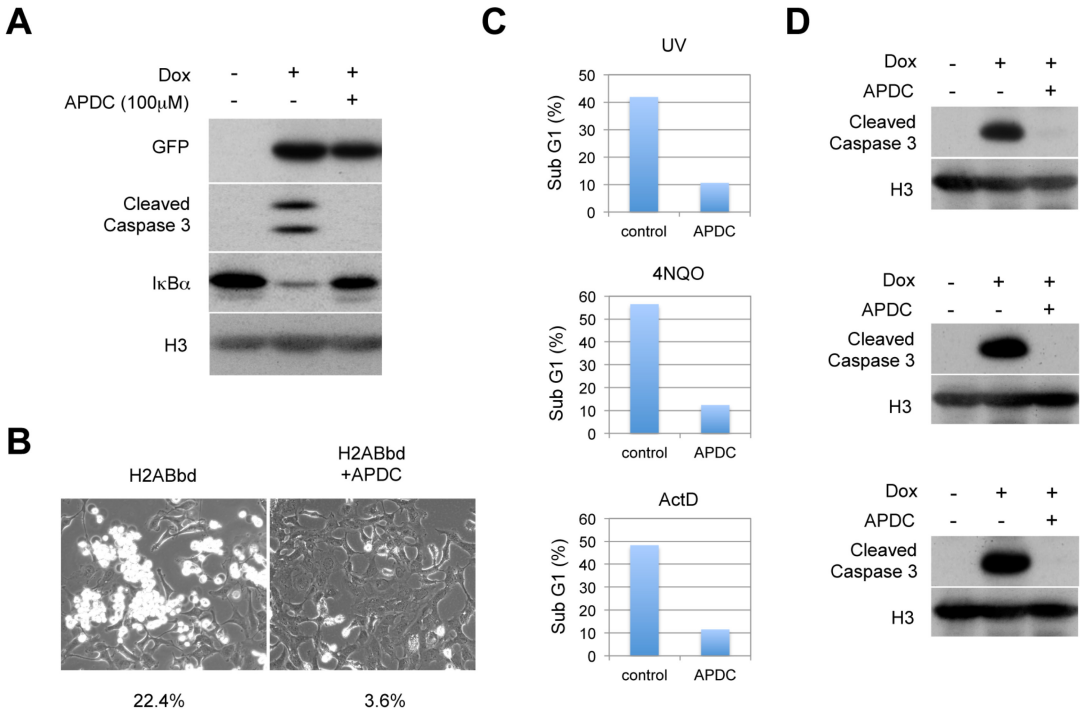

Figure S4.

H2ABbd mediated apoptosis was blocked by NF-κB inhibitor.

(A) (B) HeLa cells transfected with Dox inducible EGFP-H2ABbd were cultured in the presence or absence of Dox and APDC (100μM) for 48 hr. Total cell extracts were prepared for immunoblotting **(A)**. Typical DIC images and sub-G1 DNA contents (%) analyzed by FACS are shown **(B)**. **(C) (D)** HeLa cells were irradiated with UV (100J/m^2) or treated with 4NQO (10μM), Actinomycin D (0.1μg/ml) and cultured with or without APDC for 18 hr. sub-G1 DNA contents (%) analyzed by FACS are shown in **(C)** and total cell extracts were prepared and analyzed by immunoblotting **(D)**.

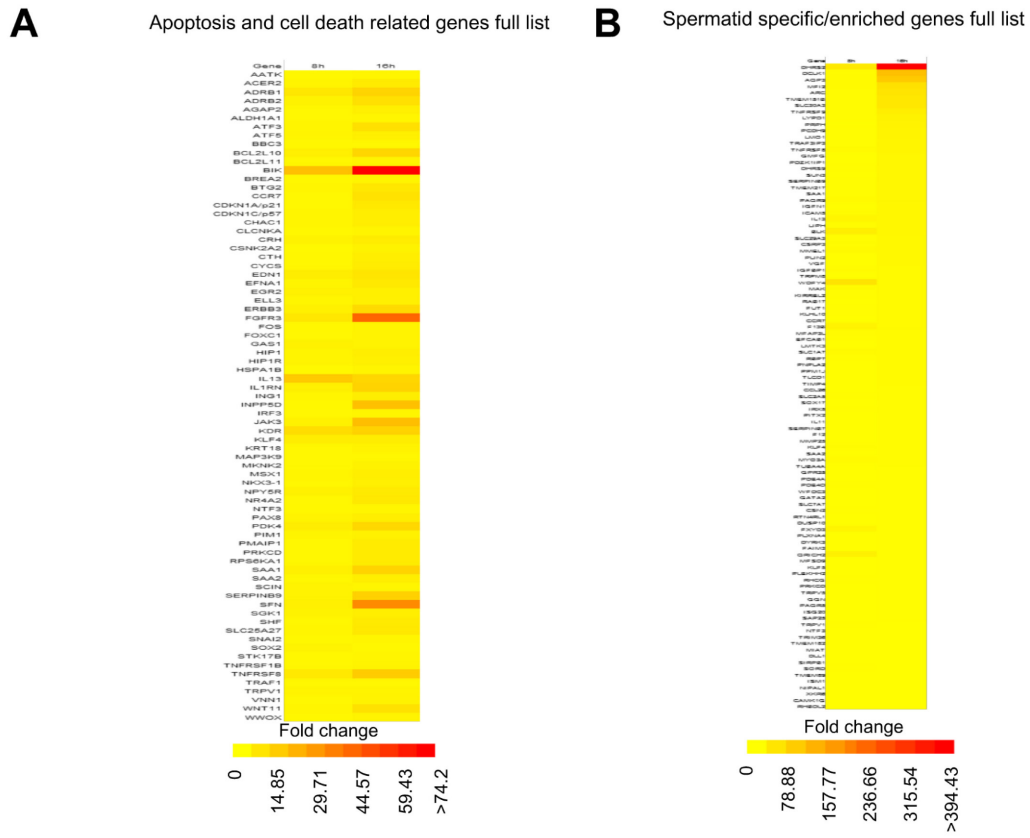

Figure S5.

Heatmaps for H2ABbd upregulated genes linked with apoptosis and germ cells specific functions. Gene expression heatmaps of all analyzed genes related in apoptosis and cell death, and specific to or enriched in spermatids that were upregulated by overexpression of H2ABbd. Genes upregulated over a 2 fold are shown.

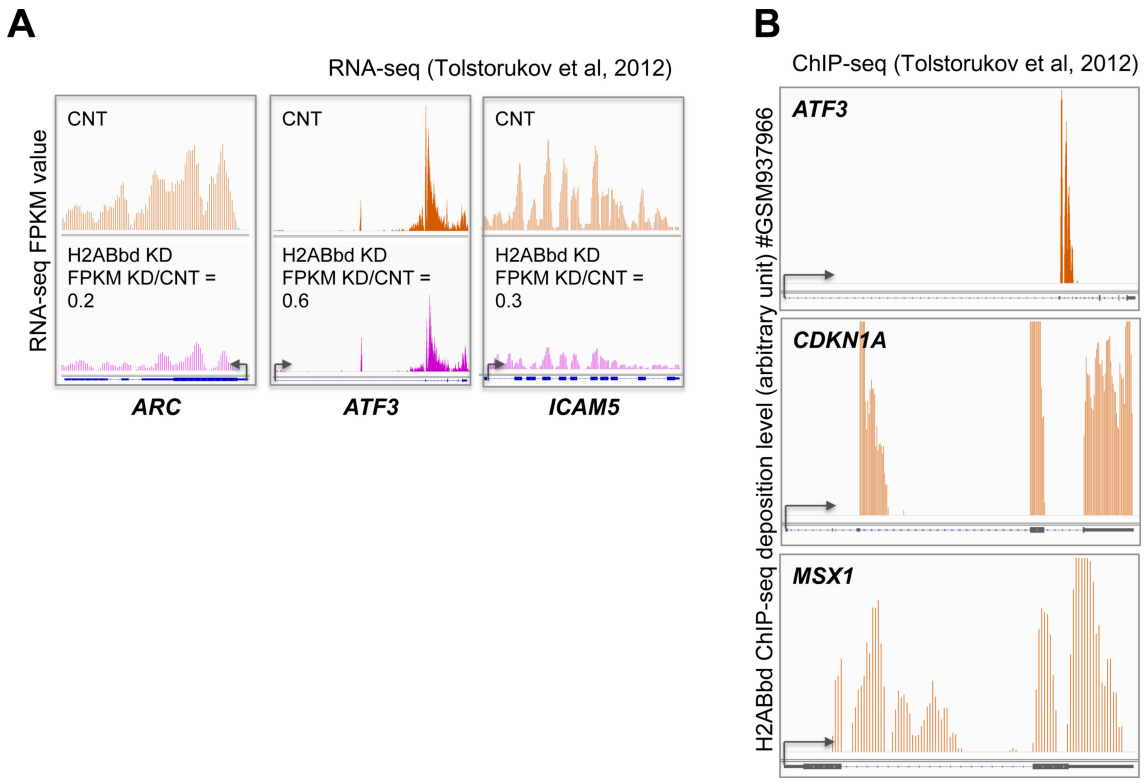

Figure S6.

Putative model for regulation of developmental genes and apoptosis related genes by H2ABbd.

(A) RNA-seq FPKM results from publicly available data (Tolstorukov et al, 2012) are shown. Top panel exhibits the expression pattern of respective genes in the control siRNA (CTL) experiment, while the bottom panel demonstrates the results after H2ABbd knockdown (KD). The FPKM ratio for knockdown/control (KD/CNT) is shown inside the box. The name of each gene locus and genomic organization is given at the bottom. Promoters are shown by arrows. **(B)** ChIP-seq deposition pattern of H2ABbd into apoptosis related gene loci such as CDKN1A, MSX1 and ATF3 were investigated by using the above-mentioned publicly available dataset. The name of each gene locus and genomic organization is given at the bottom. Promoters are shown by arrows.

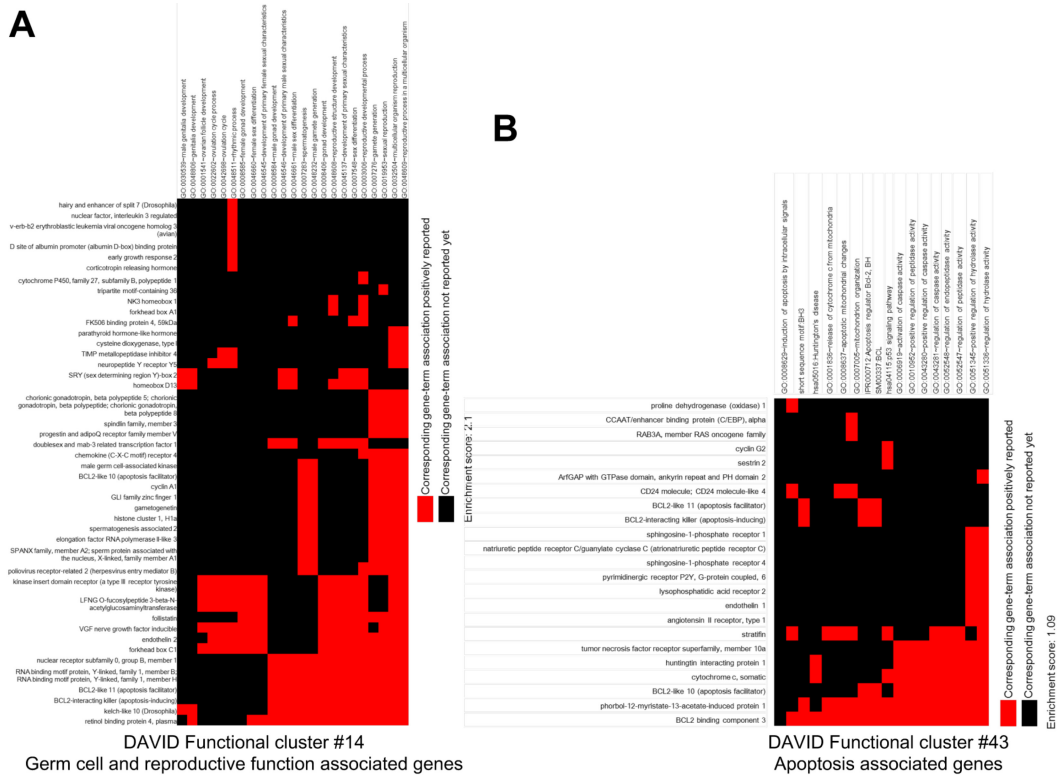

Figure S7.

Functional clustering of the genes that are upregulated upon H2ABbd overexpression.

2-D view heatmaps were generated by the DAVID analytical tools for the genes that exhibited transcriptional activation (> 2 fold) in H2ABbd ectopically expressing cells. Positive gene-term associations that passed the statistical filter assigned by the DAVID algorithm are delineated in red (value = 1), while the associations that have not been reported yet are shown in black (value = 0). Enrichment score for functional clustering is shown beside each panel. **(A)** Functional cluster (cluster 14) incorporating gene-terms associated with germ cell development and reproduction. **(B)** The right panel shows functional cluster 43, which represents gene-terms linked with apoptosis and cell death.