

## **Supplemental Information**

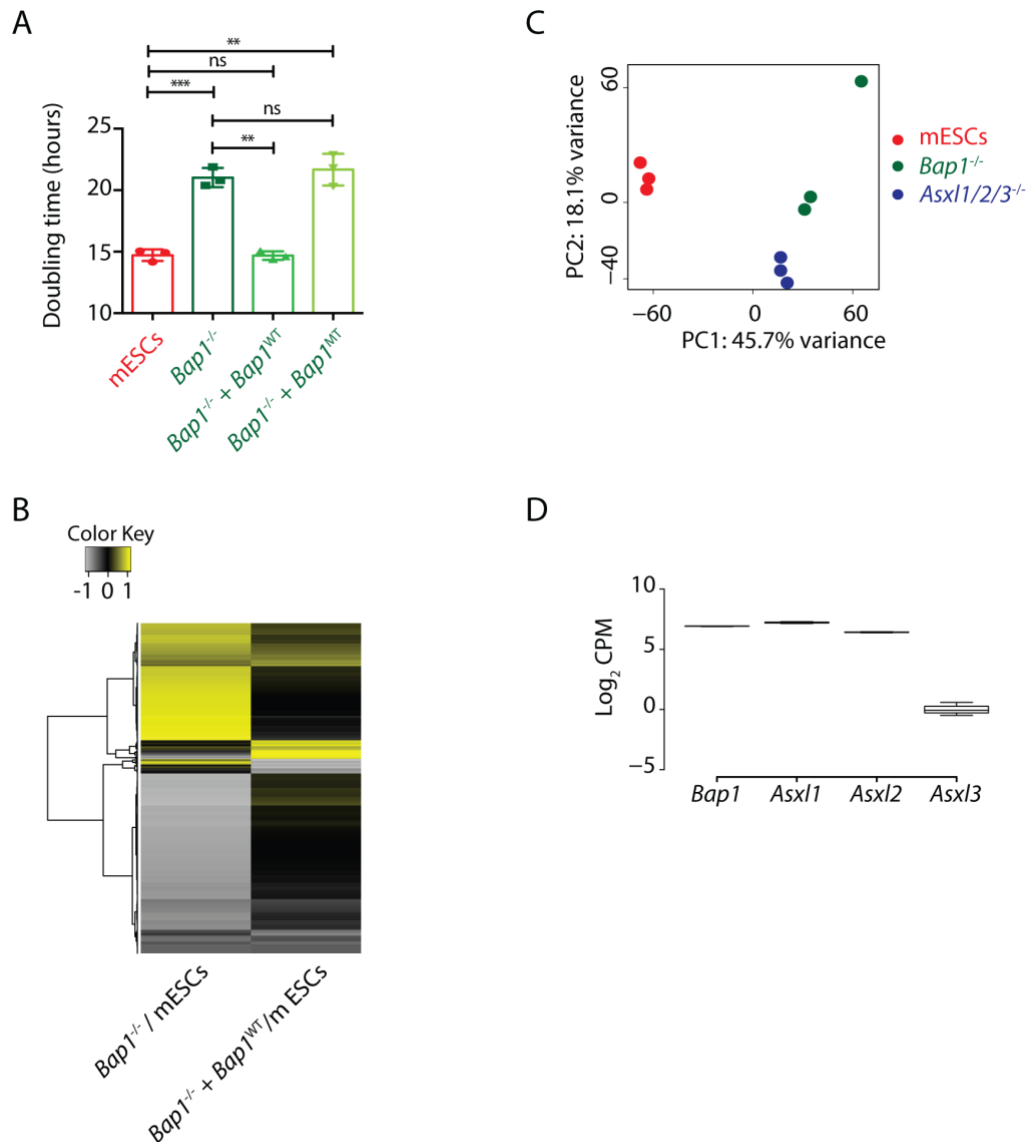
**PR-DUB maintains expression of critical genes through FOXK1/2 and ASXL1/2/3-dependent recruitment to chromatin and H2AK119ub1 deubiquitination**

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**Supplemental Figures S1-S5**



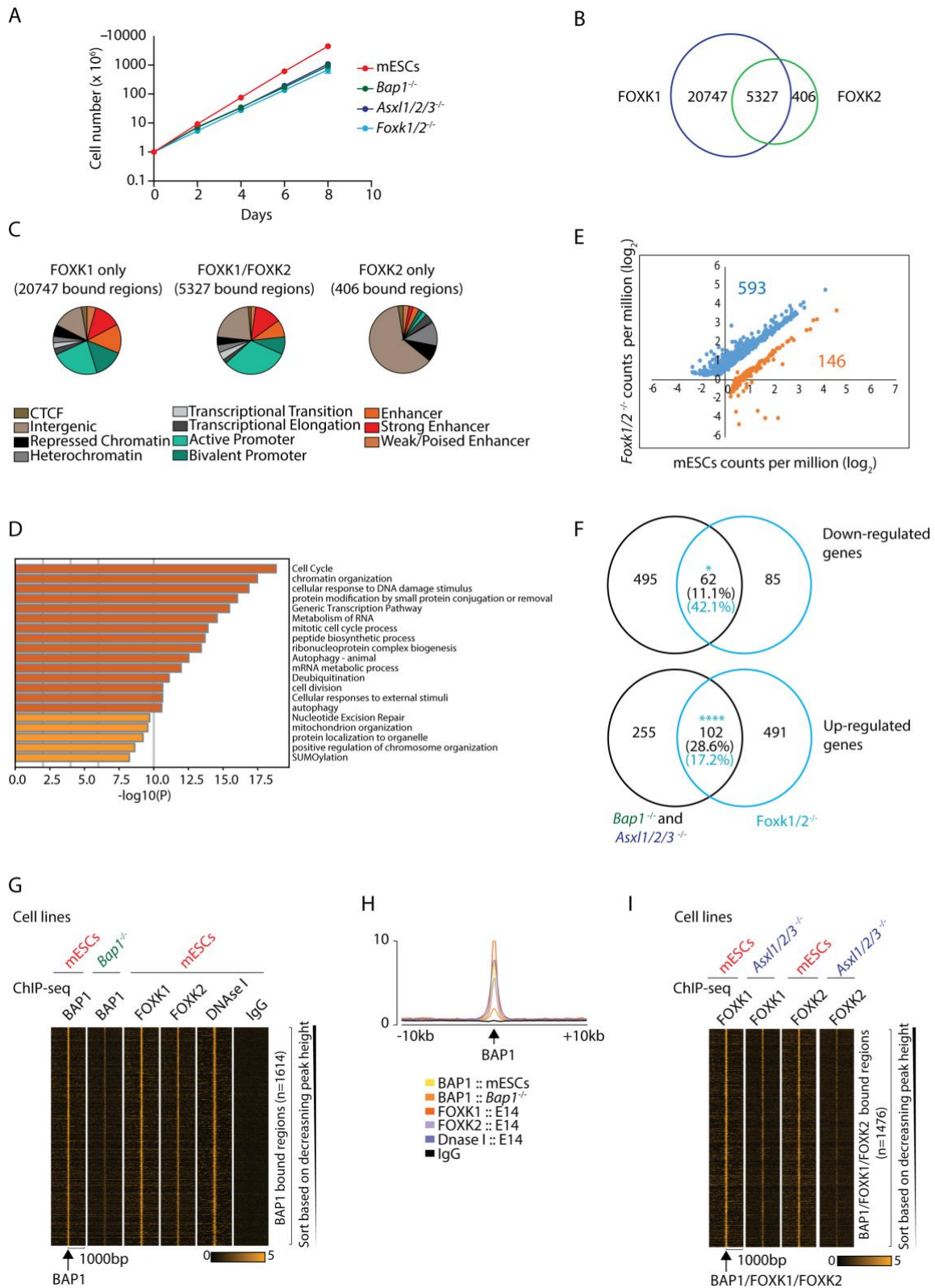
**Supplemental Figure S1: Characterization of *Bap1*<sup>-/-</sup>, *Asx1*<sup>-/-</sup>, *Asx2*<sup>-/-</sup>, *Asx3*<sup>-/-</sup> and *Asx1/2/3*<sup>-/-</sup> mESCs.**

**(A)** Doubling time (in hours) for wild type mESCs, *Bap1*<sup>-/-</sup> mESCs, *Bap1*<sup>-/-</sup> + *Bap1*<sup>WT</sup> and *Bap1*<sup>-/-</sup> + *Bap1*<sup>MT</sup> mESCs was performed in biological triplicates. Unpaired *t*-test with welsh correction. \*\*\*: *p*val < 0.0001.

**(B)** Heatmap for the log<sub>2</sub> fold gene expression changes, in *Bap1*<sup>-/-</sup> mESCs versus wild type mESCs (left) and *Bap1*<sup>-/-</sup> + *Bap1*<sup>WT</sup> versus wild type mESCs (right).

**(C)** Principal component analysis of RNA-seq for three biological replicates for wild type ESCs, *Bap1*<sup>-/-</sup> mESCs and *Asx1/2/3*<sup>-/-</sup> mESCs.

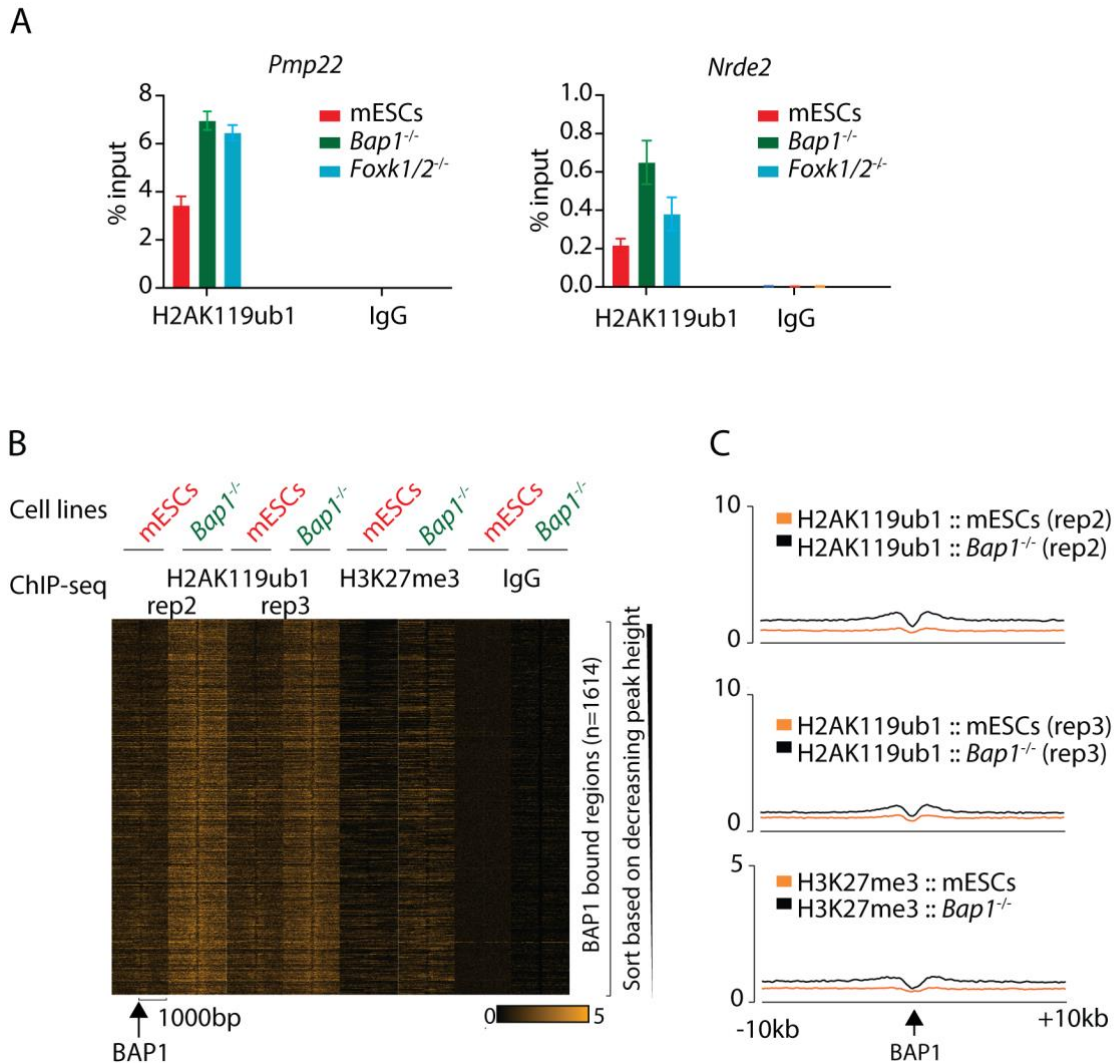
**(D)** Gene expression levels of *Bap1*, *Asx1*, *Asx2* and *Asx3* in wild type mESCs showing the log<sub>2</sub> CPM from three biological replicates for each gene.



## Supplemental Figure S2: Characterization of *Foxk1*<sup>-/-</sup>, *Foxk2*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs.

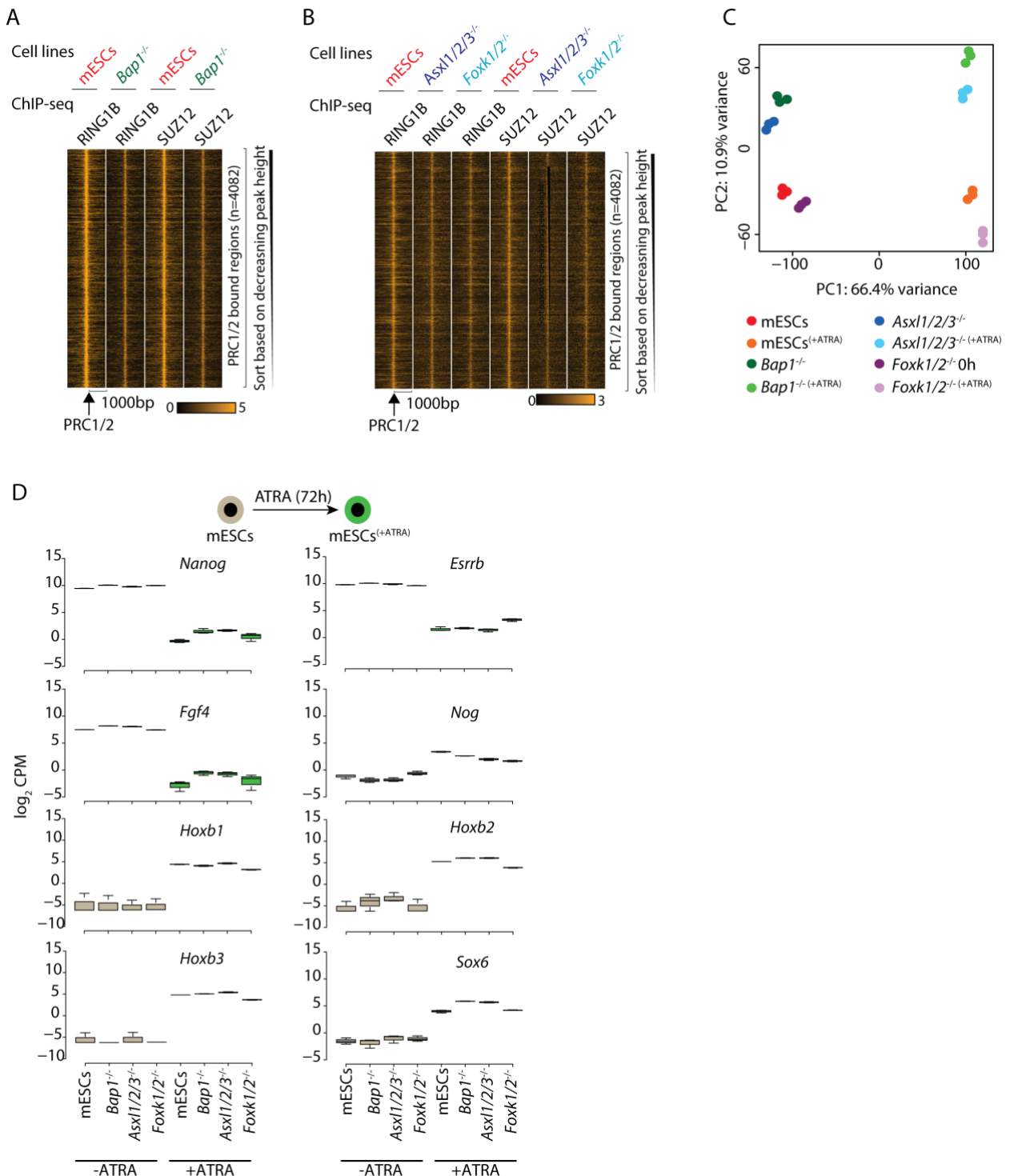
(A) Cell proliferation of wt mESCs, *Bap1*<sup>-/-</sup> mESCs, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs was performed in three independent biological triplicates per condition.

- (B)** Euler diagram showing the overlap of FOXK1 and FOXK2 binding in mESCs.
- (C)** Pie diagrams showing a Hidden Markov analysis of FOXK1 only (20750), FOXK1/2 (5327) and FOXK2 only (406) bound positions in wt mESCs. The organization of these regions into 11 genomic categories is shown in the lower part of the panel.
- (D)** The most significant GO terms for the 5327 FOXK1/2 genes bound by both transcription factors
- (E)** Gene expression analysis of the indicated cell lines. Log<sub>2</sub>-normalized mean counts of mapped reads in *Foxk1/2*<sup>-/-</sup> mESCs versus wt mESCs. Only genes up- (blue) and down-regulated (orange) are shown. Down-regulated genes in *Foxk1/2*<sup>-/-</sup> mESCs were defined with the following criteria: log<sub>2</sub> fold change ≤ -1, *pval* ≤ 0.05, log<sub>2</sub> CPM in mESCs ≥ 0.5. Up-regulated genes in *Foxk1/2*<sup>-/-</sup> mESCs were defined with the following criteria: log<sub>2</sub> fold change ≥ 1, *pval* ≤ 0.05, log<sub>2</sub> CPM in KO ≥ 0.5.
- (F)** Euler diagrams demonstrating up-regulated (left) and down-regulated (right) genes in common between *Bap1*<sup>-/-</sup> and *Asx1/2/3*<sup>-/-</sup> mESCs versus wt mESCs. Fisher's exact test , \*\*\*\*: *pval* < 0.0001, \*\*: *pval* = 0.0102.
- (G)** Heat map illustrating the signal of indicated ChIP-seq profiles (BAP1, FOXK1, FOXK2 and DNase I) in wt ESCs and *Bap1*<sup>-/-</sup> mESCs in 2Kb around the BAP1 peaks.
- (H)** Enrichment profiles of BAP1, FOXK1, FOXK2 and DNase I demonstrate for a 20 kb region around the center of the BAP1 binding peaks.
- (I)** Heatmap illustrating the signal of indicated ChIP-seq profiles (FOXK1 and FOXK2) in wt ESCs and *Asx1/2/3*<sup>-/-</sup> mESCs in 2Kb around the BAP1/FOXK1/FOXK2 peaks.



**Supplemental Figure S3: H2AK119ub1 and H3K27me3 binding profiles in wt and *Bap1*<sup>-/-</sup> mESCs.**

- (A)** ChIP-qPCR profiles of H2AK119ub1 and IgG (% input) in two loci (*Pmp22* and *Nrde2*) for wild type, *Bap1*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs.
- (B)** Heat maps illustrating the enrichments of H2AK119ub1 (replicate 2 and 3) and H3K27me3 in 2 kb centered around the peaks of BAP1-bound regions in wild type and *Bap1*<sup>-/-</sup> mESCs.
- (C)** Average profile of the H2AK119ub1 (replicate 2 and 3) and H3K27me3 ChIP-seq signal for a 20 kb region around the center of the BAP1 binding peaks.



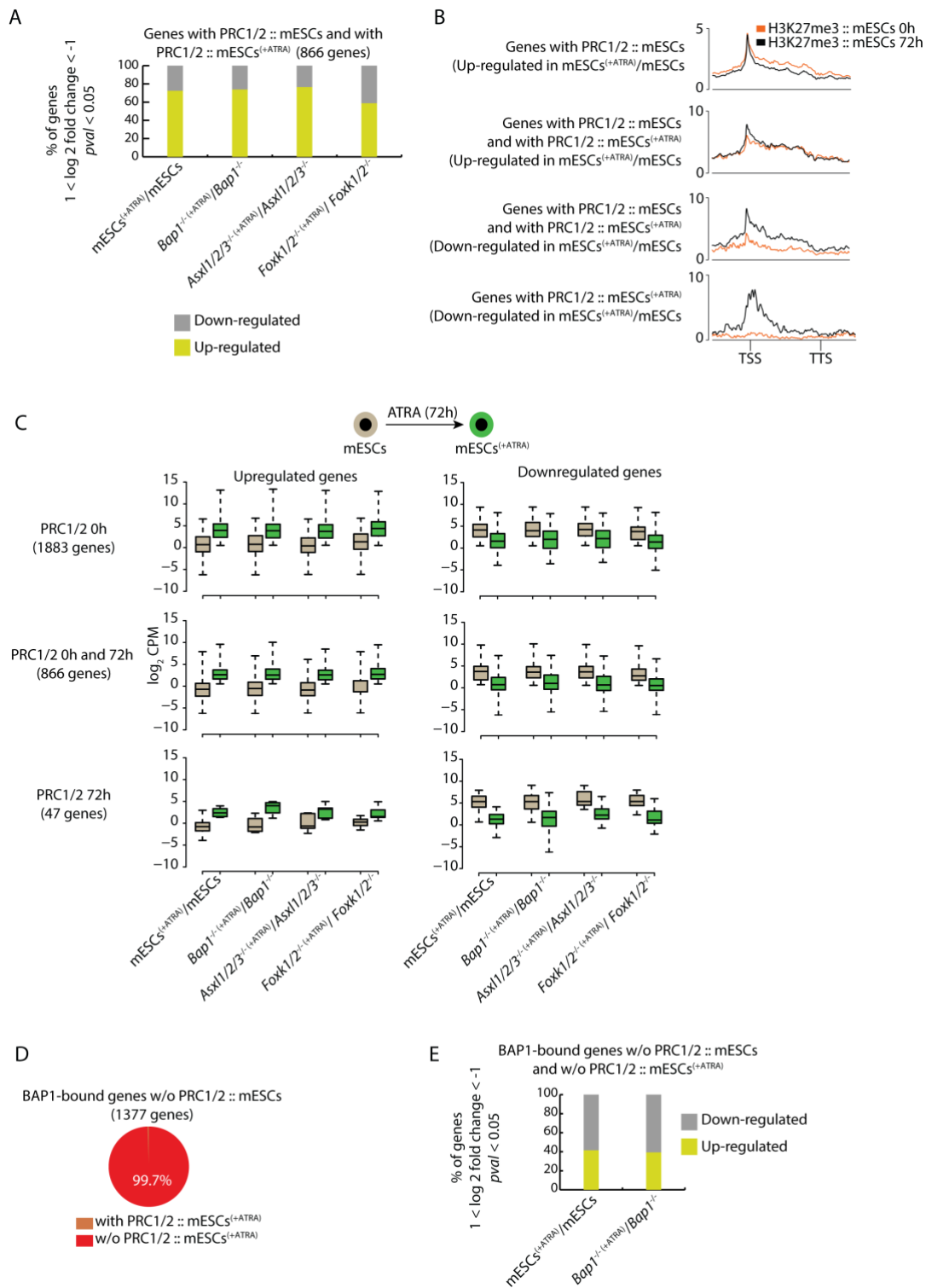
**Supplemental Figure S4: Binding profile of PRC1/2 upon *Bap1* deletion.**

**(A)** Heat map illustrating the enrichments of RING1B and SUZ12 in wild type mESCs and *Bap1*<sup>-/-</sup> mESCs for a 2 kb region centered around the peaks of PRC1/2 bound regions.

**(B)** Heat map illustrating the enrichments of RING1B and SUZ12 in wild type mESCs, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs for a region centered 2 kb around the peaks of

PRC1/2 bound regions.

- (C) Principal component analysis of RNA-seq for three biological replicates for wild type, *Bap1*<sup>-/-</sup>, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs without (0h) and upon (72h) ATRA induced differentiation.
- (D) Log<sub>2</sub> CPM values of various genes in wild type, *Bap1*<sup>-/-</sup>, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs without (0h) and with (72h) ATRA treatment.



## Supplemental Figure S5: Role of BAP1 in regulating transcription during ATRA-induced differentiation.

**(A)** Expression changes of genes in response to ATRA-induced differentiation in wild type, *Bap1*<sup>-/-</sup>, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs. The panel shows the % of the 866 genes that



are bound by PRC1/2 in untreated mESCs and retain PRC1/2 upon ATRA treatment and are up- or down-regulated in response to ATRA.

- (B)** The average signal (in metagenes) of H3K27me3 in non-treated (orange) and ATRA treated (black) mESCs, for various sets of genes, as described left from each panel.
- (C)** Log<sub>2</sub> CPM values of the genes which are bound by PRC1/2 in proliferating ESCs, of the genes bound by PRC1/2 with and without ATRA treatment and of the genes bound by PRC1/2 only in ATRA-treated cells and separated in up-regulated (left panels) and down-regulated genes in wild type, *Bap1*<sup>-/-</sup>, *Asx1/2/3*<sup>-/-</sup> and *Foxk1/2*<sup>-/-</sup> mESCs in response to 72 hours ATRA treatment.
- (D)** Pie chart depicting the percentage of the 1377 genes bound by BAP1 and without PRC1/2 in proliferating (untreated) mESCs, illustrating the proportion that gains or lose PRC1/2 binding in response to 72 hours ATRA treatment.
- (E)** The bar graphs show the percentage of BAP1-bound genes and without PRC1/2 in proliferating (untreated) mESCs, that are up- or down-regulated in response to 72 hours treatment with ATRA in wild type and *Bap1*<sup>-/-</sup> mESCs.