Supplementary Information

Genome-wide profiling of nardilysin target genes reveals its role in epigenetic regulation and cell cycle progression

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Fig.S1

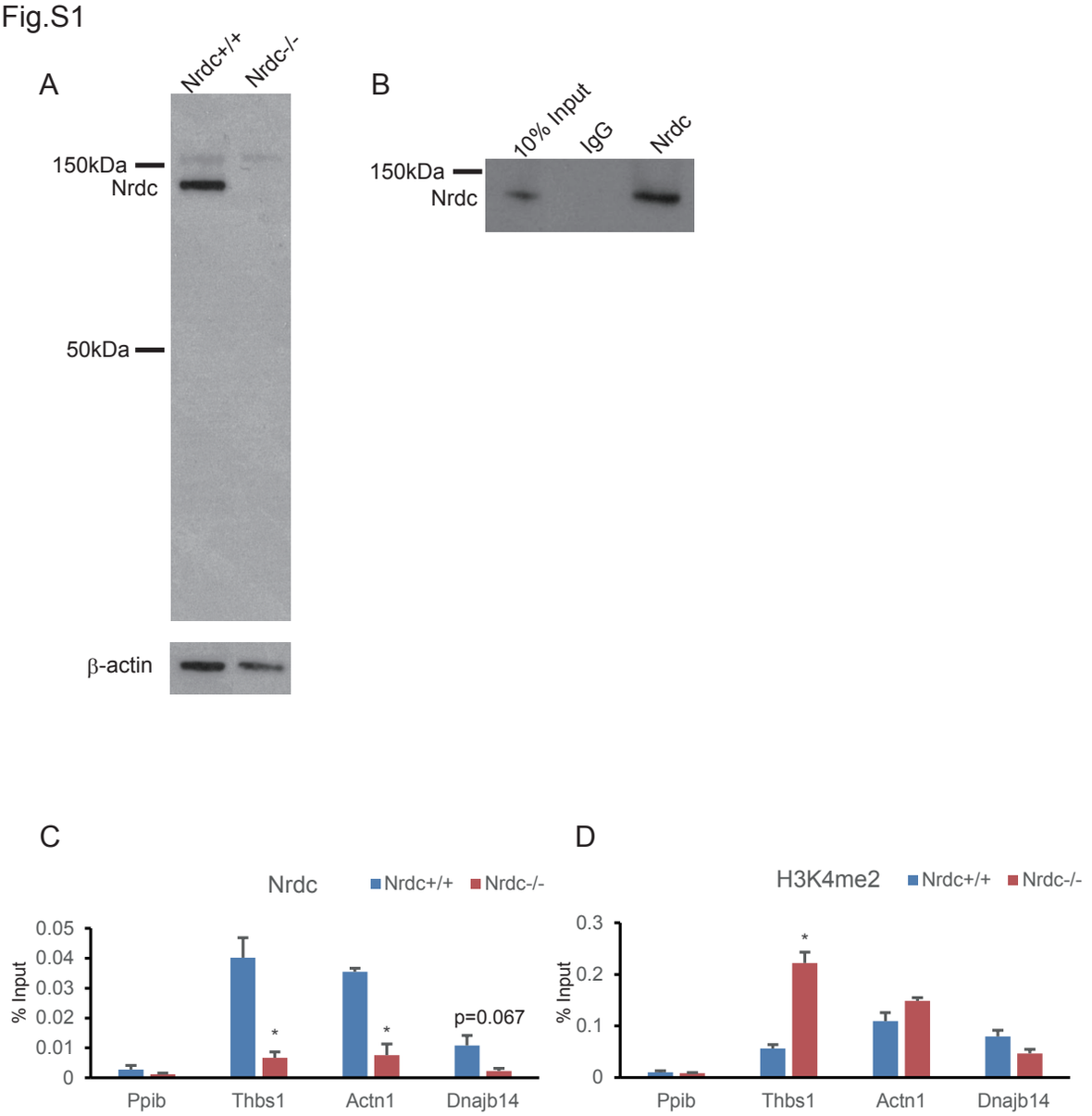


Fig. S1. Validation of a mouse monoclonal anti-Nrdc antibody (#2E6) for ChIP-seq.

(A) A Western blot analysis of cross-linked chromatin samples from Nrdc+/+ and Nrdc-/- iMEF shows the high specificity of the anti-Nrdc antibody. (B) Samples obtained by immunoprecipitation with the anti-Nrdc antibody (#2E6) or control IgG of cross-linked chromatin samples from Nrdc+/+ iMEF were probed with the anti-Nrdc-antibody (#135).

(C, D) ChIP-PCR analysis using anti-Nrdc (C) and anti-H3K4me2 (D) antibody in Nrdc+/+ and Nrdc-/- iMEF (n=3 per group). Target genes (Thbs1, Actn1, Dnajb14) and negative control gene (Ppib) were randomly selected from H3K4me2-enriched and H3K4me2-negative genome regions, respectively.

* indicates $p < 0.05$ between Nrdc+/+ v.s. Nrdc-/- group.

Fig.S2

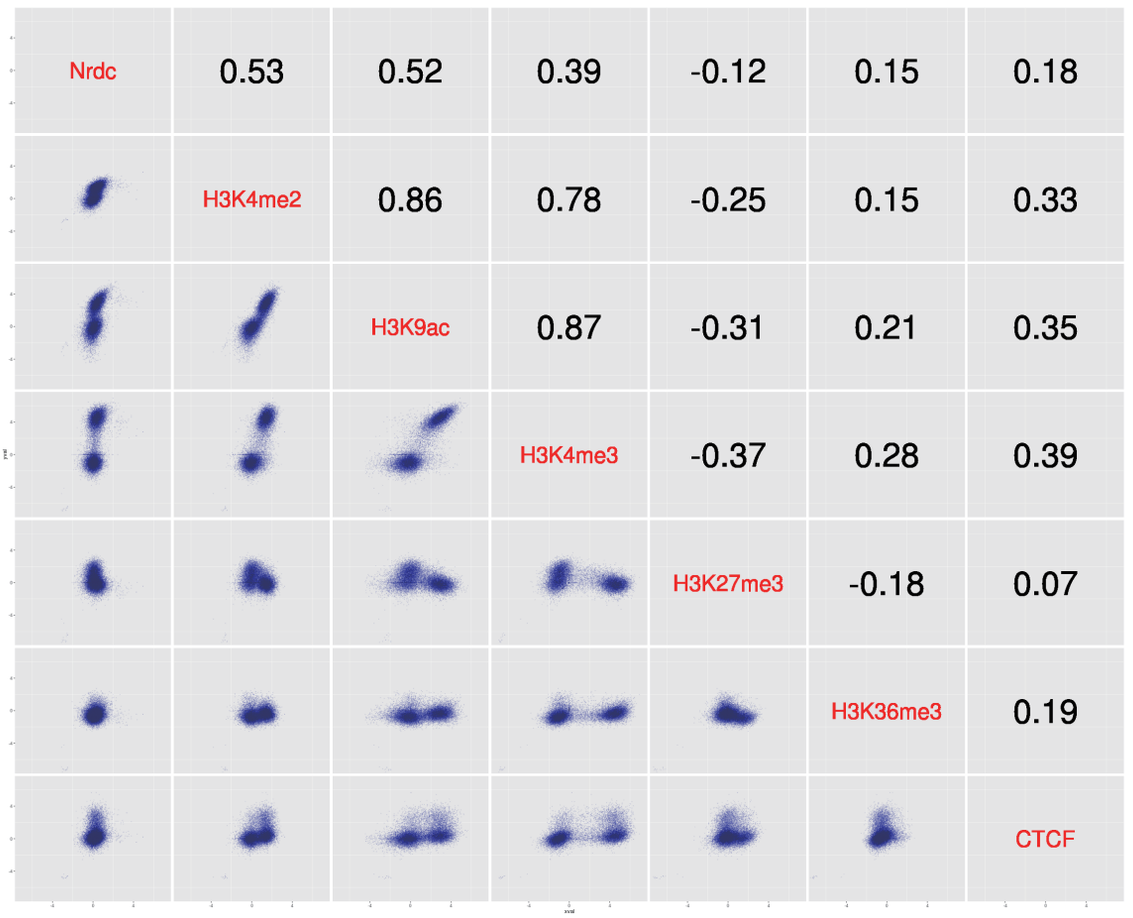


Fig. S2. Correlation analysis of the enrichment of Nrdc and histone marks in iMEF.

Correlation analysis of the relative enrichment of Nrdc and indicated histone marks (H3K4me2, H3K9ac, H3K4me3, H3K27me3, H3K36me3, and CTCF transcriptional factor) in promoters of Ensembl genes in Nrdc+/+ iMEF. Pearson's r values are shown.

Fig.S3

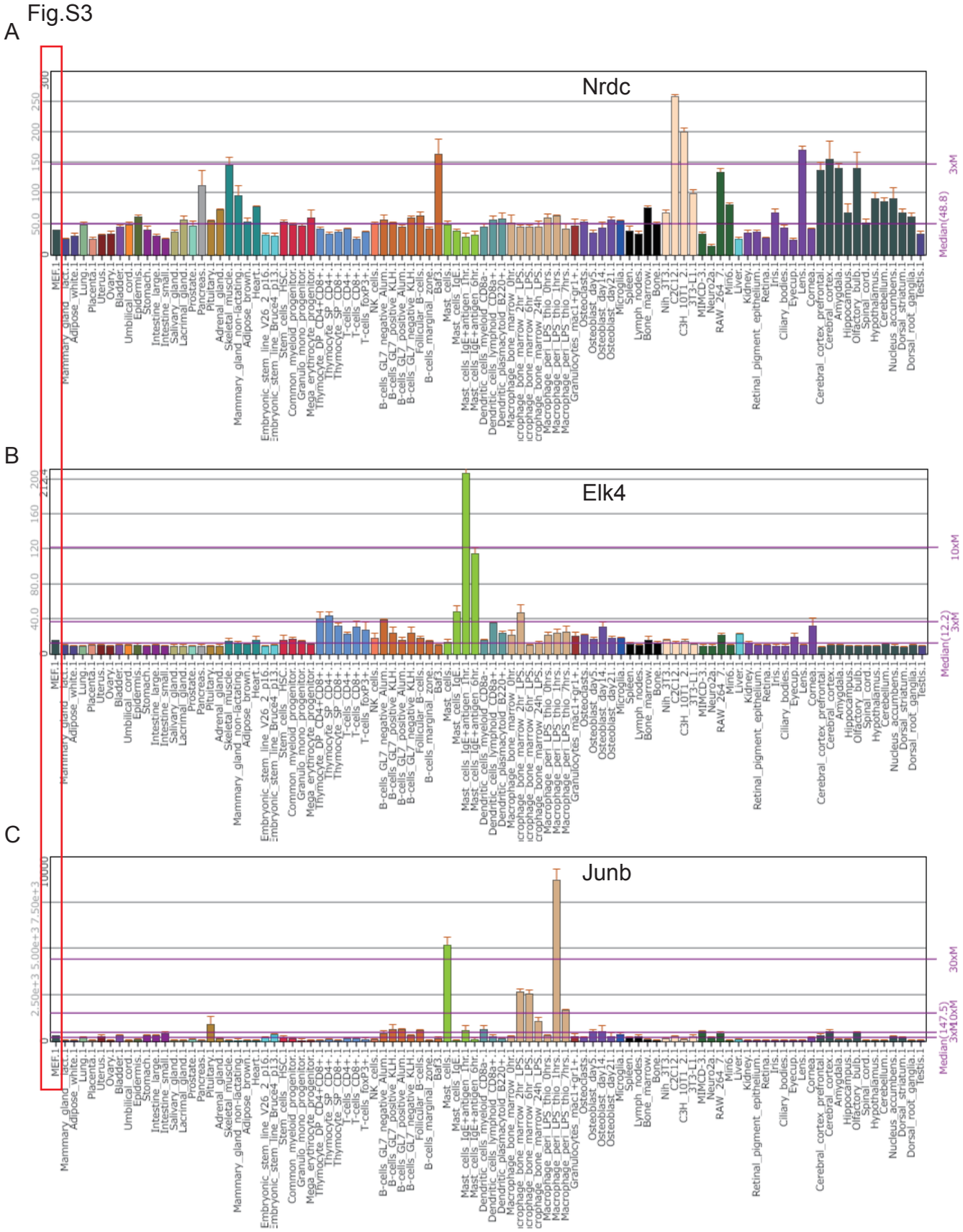


Fig. S3. Expression profiles of Nrdc, Elk4, and Junb in mice tissues from BioGPS. (A) Nrdc is ubiquitously expressed in mice tissues and cell lines. (B, C) Elk4 and Junb are specifically expressed in mast cells and macrophages, respectively.

Fig.S4

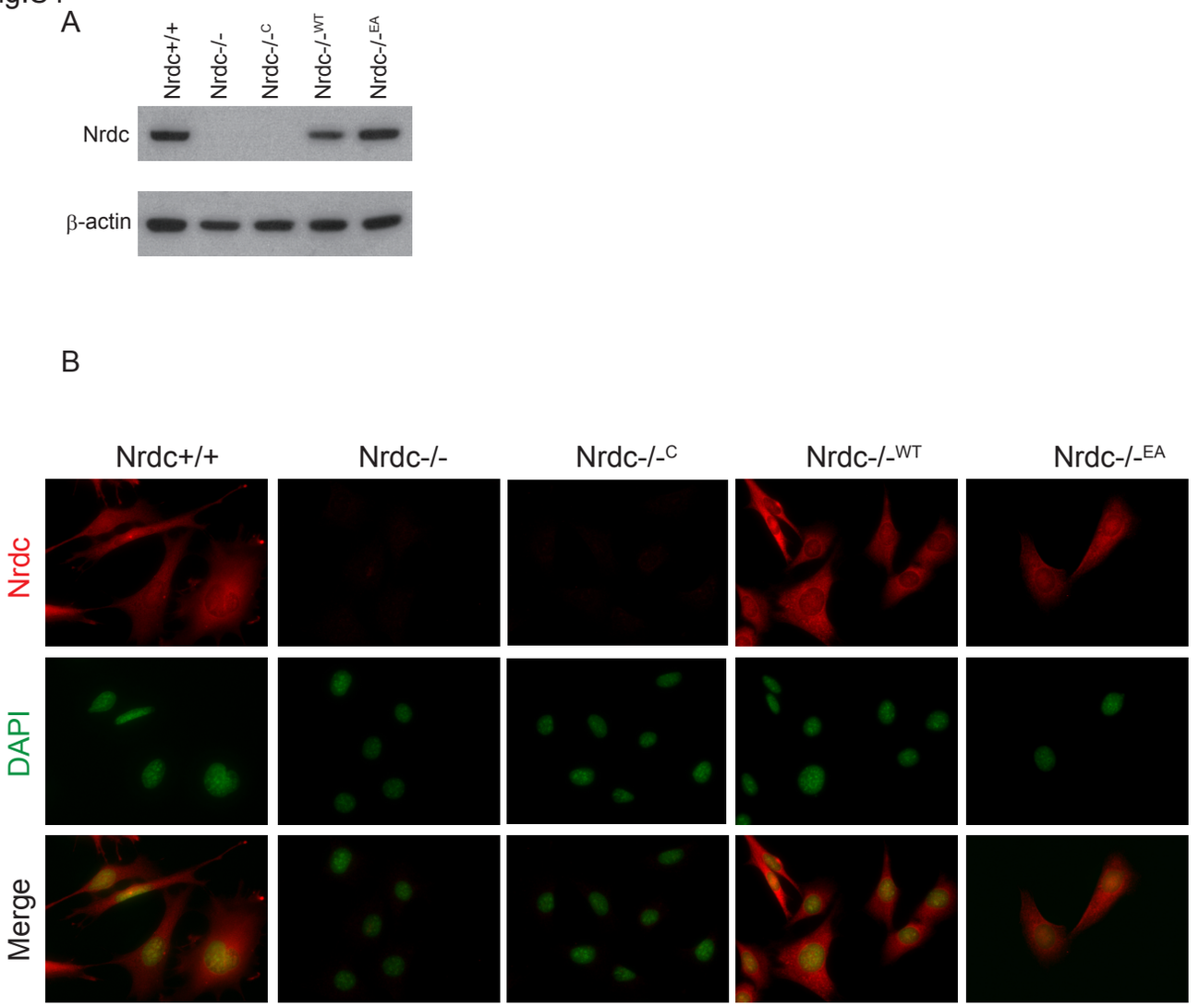
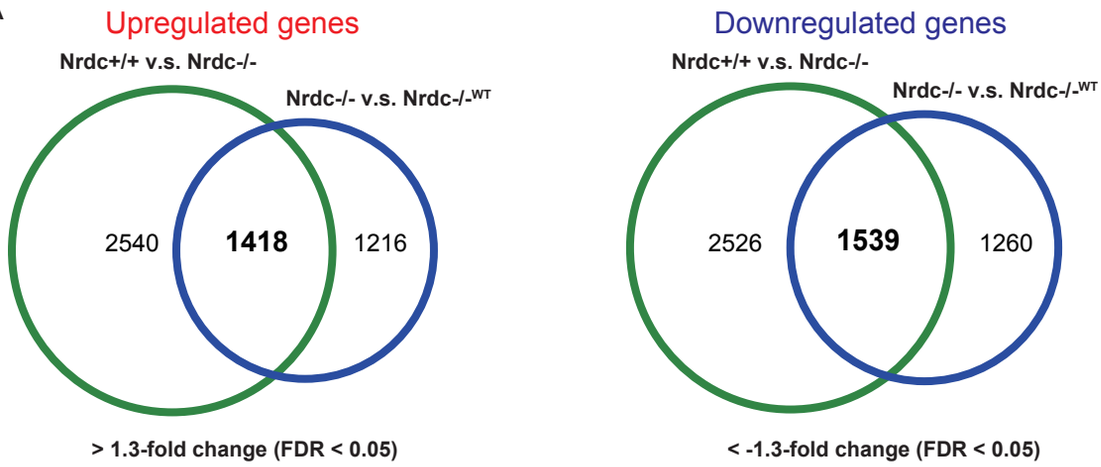


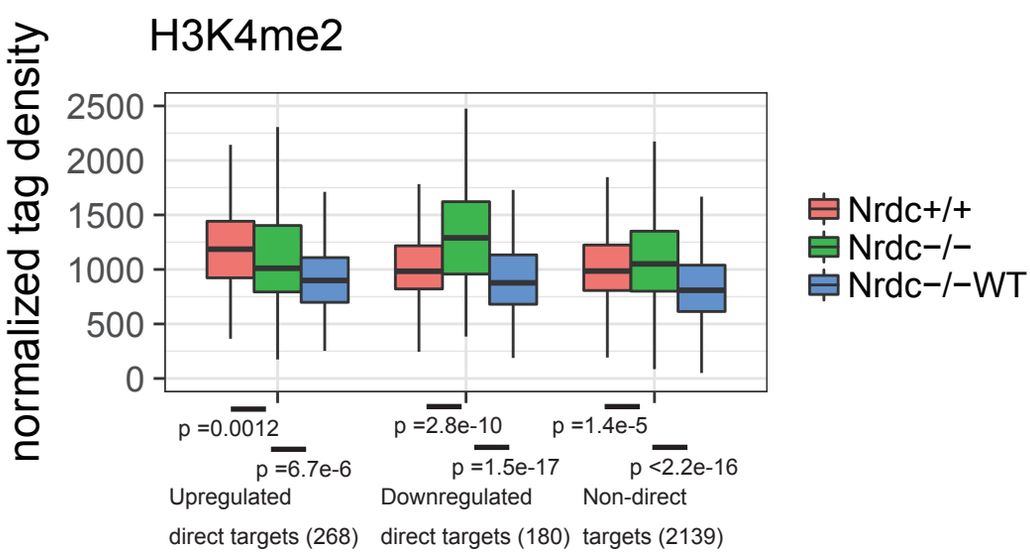
Fig. S4. Validation of Nrdc protein expression in each iMEF.

(A) Western blotting showing the protein levels of Nrdc and β-actin in Nrdc+/+, Nrdc-/-, Nrdc-/-^{C}, Nrdc-/-^{WT}, and Nrdc-/-^{EA} iMEF. (B) Immunocytochemistry of Nrdc in each iMEF showing the similar localization pattern of Nrdc in Nrdc+/+, Nrdc-/-^{WT}, and Nrdc-/-^{EA} iMEF. DAPI was used to visualize the nucleus.

Fig.S5 A



B



C

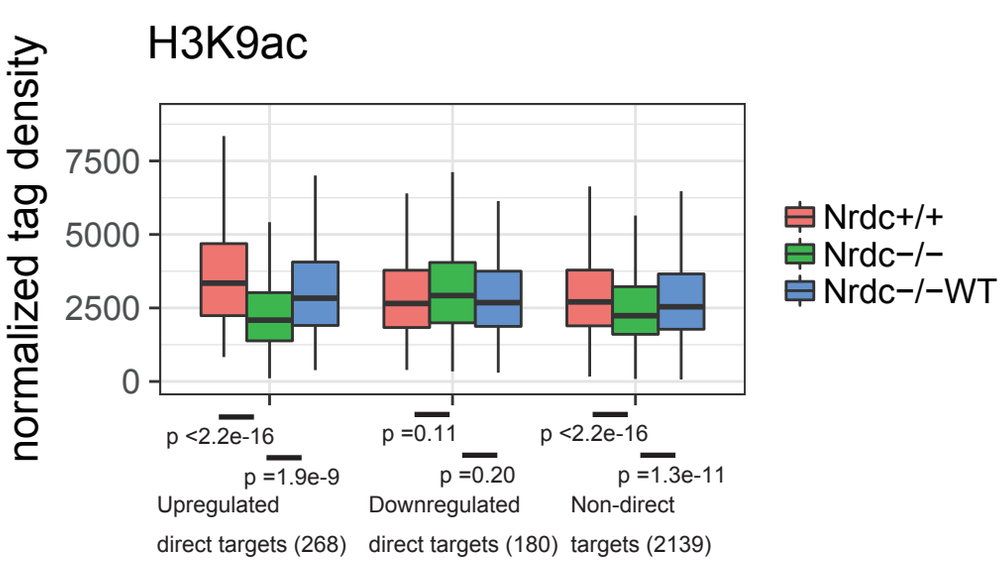


Fig. S5. Separate Analyses of histone modifications in the promoter of genes up-regulated or down-regulated by Nrdc.

(A) Separate Venn diagrams for genes up-regulated (left) or down-regulated (right) by Nrdc expression (Separate analysis of Venn diagram shown in Figure 2C).

(B, C) Separate analysis of H3K4me2 (B) and H3K9ac (C) levels in the promoters of i) up-regulated direct targets (268), ii) down-regulated direct targets (180), and iii) non-direct targets (2139).

Fig.S6

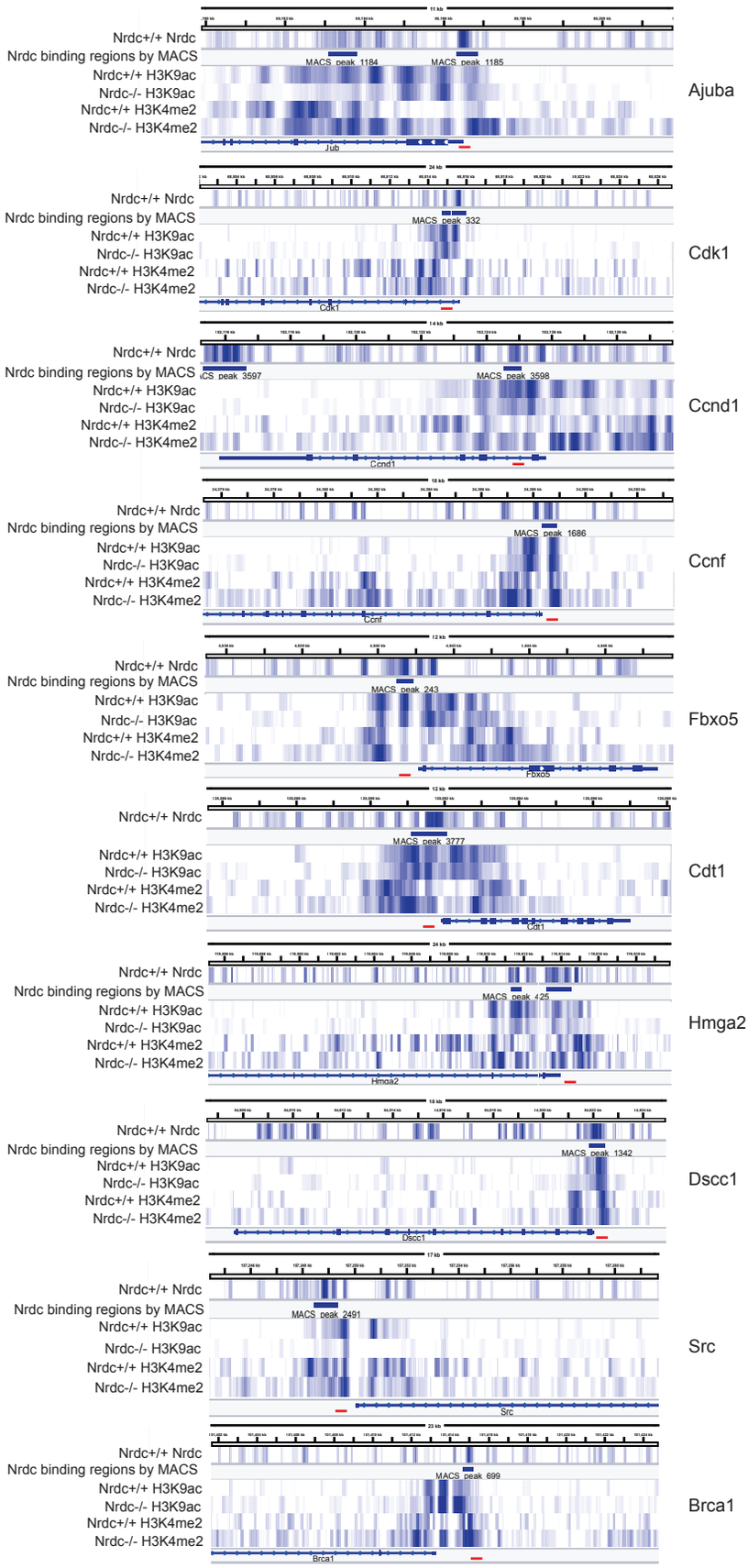
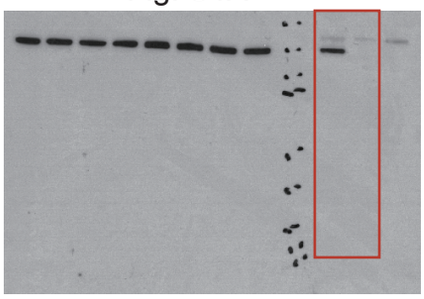


Fig.S6. Heatmaps of Nrdc, H3K4me2, and H3K9ac binding regions.

(A) Heatmaps showing the distribution of Nrdc, H3K4me2, and H3K9ac ChIP-seq signals around the Nrdc direct target promoters in Nrdc+/+ and Nrdc-/- iMEF. Nrdc binding regions detcted by MACS were also shown. Red bars indicate the specific regions for primers in ChIP-PCR experiments described in Fig. 4C-G. The color scale for the heatmap is identical between Nrdc+/+ and Nrdc-/- groups.

Fig.S7

Fig. S1A



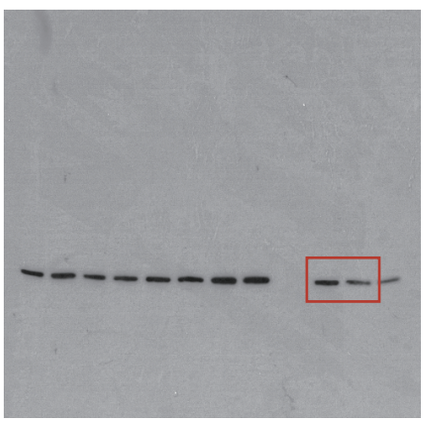


Fig. S1B

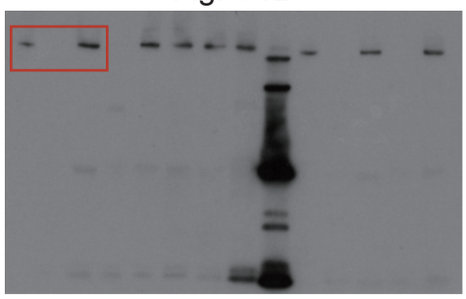
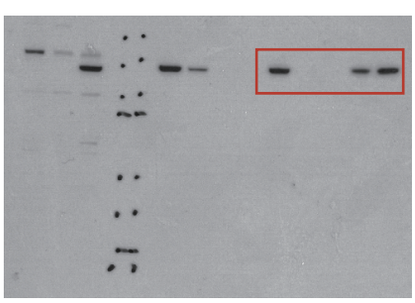


Fig. S4A



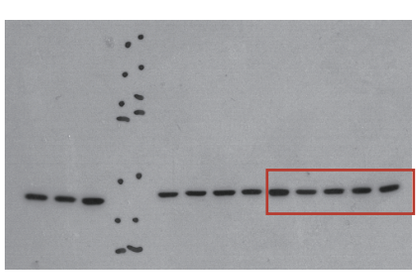


Fig. S7. Uncropped gels and western blots.
The original immunoblot data that were acquired by X-ray films are shown (Fig. S1 and S4).