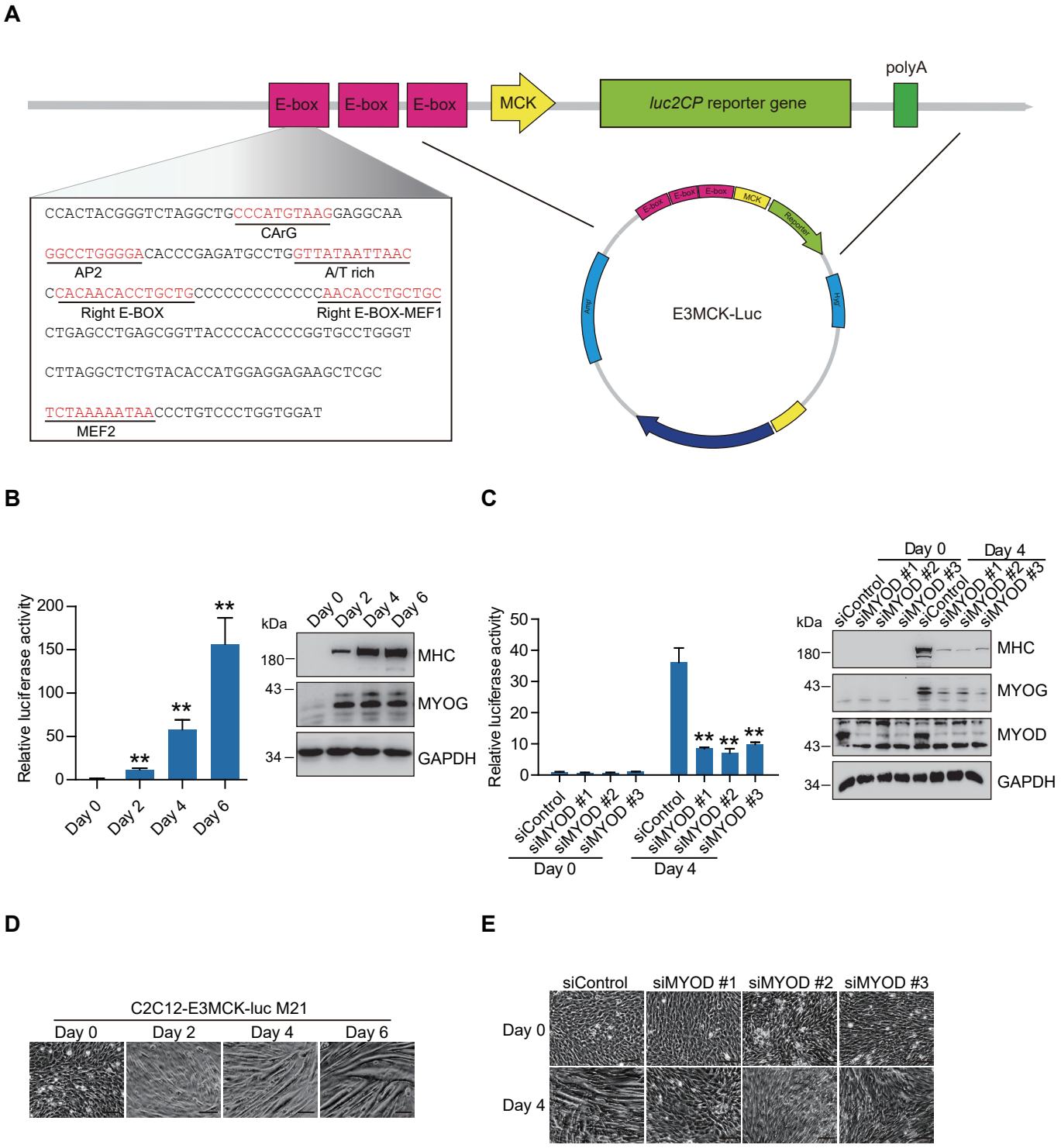
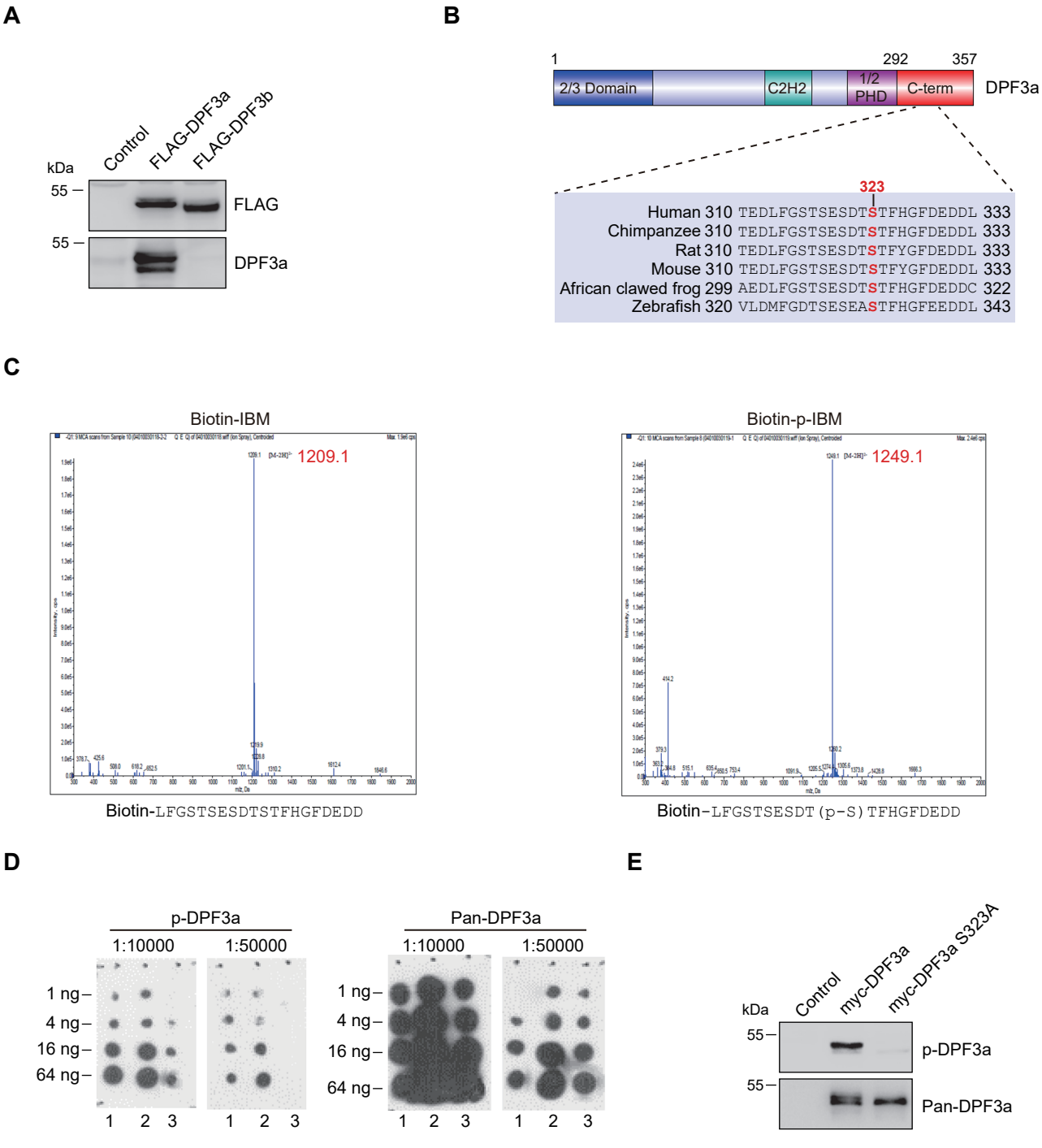
Supplementary Figure S1



Supplementary Figure S1. Construction of luciferase reporter system for epigenetic regulator screening. (A) Schematics of E3MCK luciferase reporter used in epigenetic regulator screening. **(B)** Luciferase activity quantification (left) and western blotting analysis of MYOG, MHC and GAPDH (right) in C2C12-E3MCK-luc M21 cells at various differentiation stages as indicated. Data represent as means ± SD of three replicates. ** $P < 0.01$. **(C)** Luciferase activity quantification (left) and western blotting analysis of MYOG, MHC, MYOD and GAPDH (right) in C2C12-E3MCK-luc M21 myoblasts and myotubes transfected with scramble (siControl) and three different MYOD siRNAs. **(D)** Phase contrast microscopy images of C2C12-E3MCK-luc M21 cells at various differentiation stages as indicated. Scale bars, 100 μm. **(E)** Phase contrast microscopy images of C2C12-E3MCK-luc M21 myoblasts and myotubes transfected with scramble (siControl) and three different MYOD siRNAs. Scale bars, 100 μm.

Supplementary Figure S2

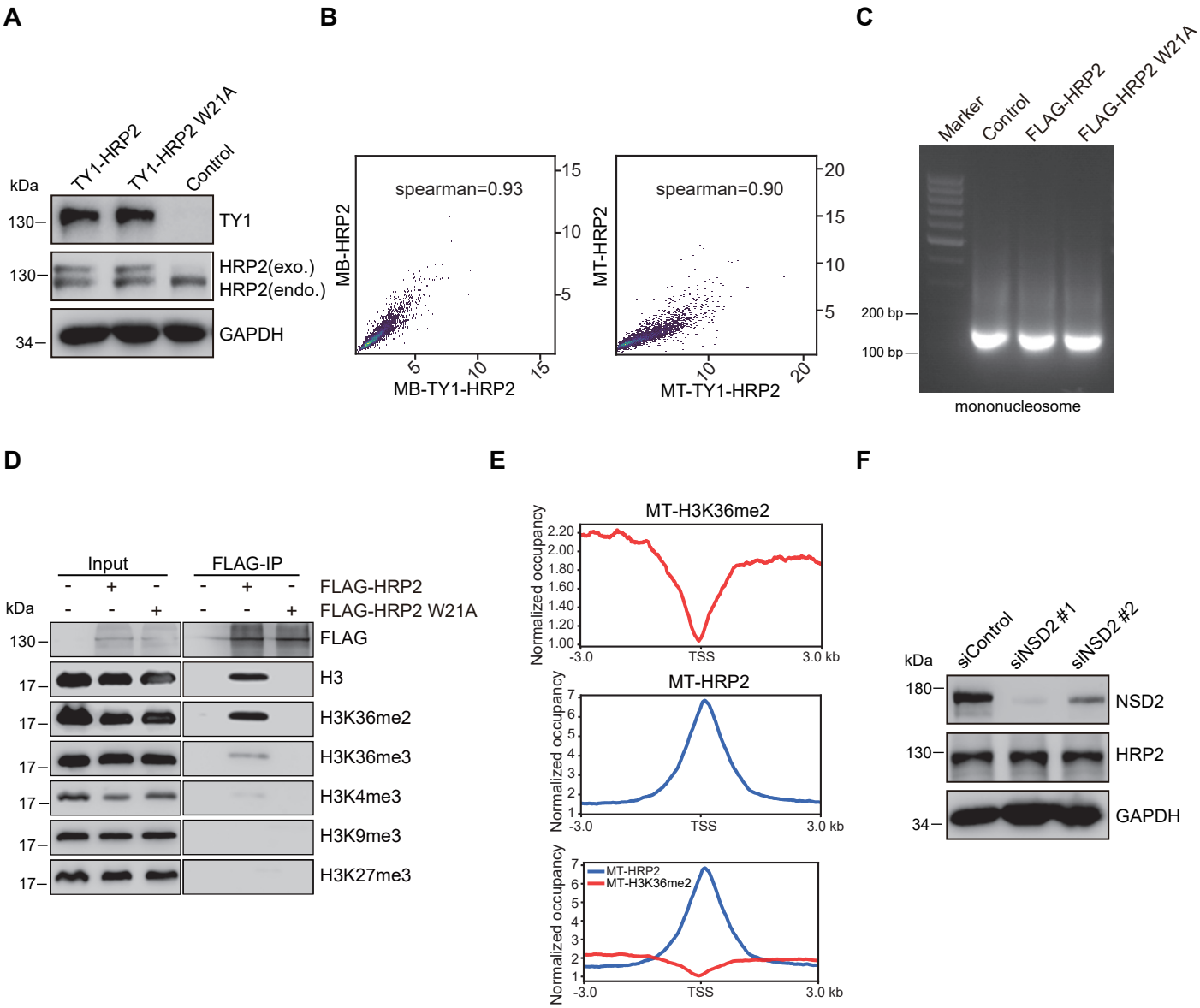


The peptide sequence is listed below:

NO.	Ag Name	Peptide Sequence
1	Phosph-DPF3(S323) peptide1	TSESDT-(phospho)S-TFHGFC
2	Phosph-DPF3(S323) peptide2	CESDT-(phospho)S-TFHGFDEDDL
3	Unmodified-DPF3(S323) peptide3	CESDTSTFHGFDEDDL

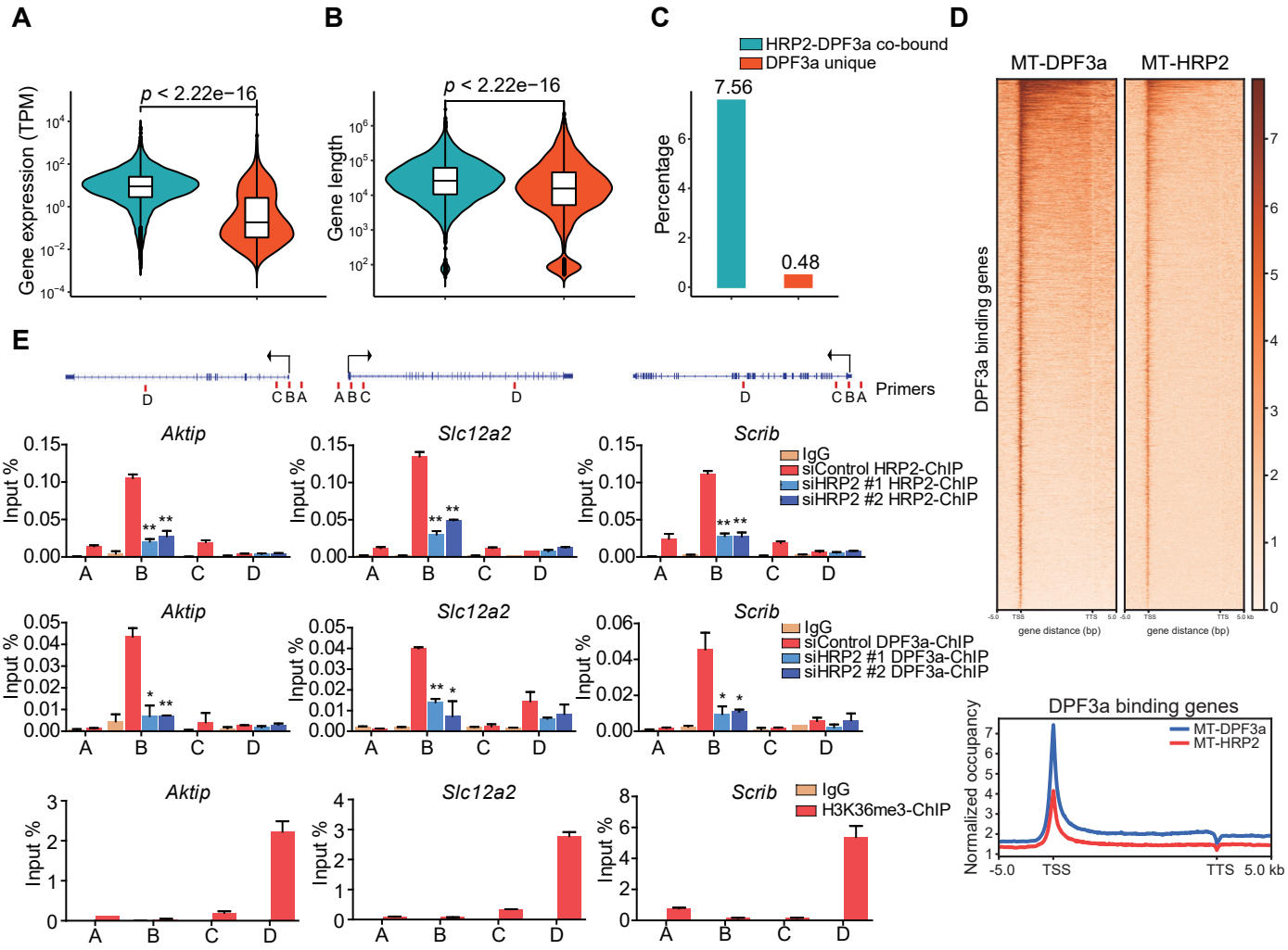
Supplementary Figure S2. DPF3a-derived peptides and antibodies validation. (A) 293T cells were transfected with FLAG-DPF3a and FLAG-DPF3b. Western blotting analysis of FLAG and DPF3a shows that homemade DPF3a antibody specifically recognizes DPF3a. **(B)** Cross-species amino acid sequence alignment of DPF3a C-terminus shows that DPF3a S323 is evolutionarily conserved. **(C)** Validation of DPF3a C-terminal peptides by mass spectrometry. Peaks with m/z values of 1209.1 and 1249.1 were respectively attributed to the non- and phosphorylated forms of the peptide. **(D)** Validation of specificity of DPF3a phospho-antibody. Dot blot analysis shows that phospho-antibody specifically recognizes phosph-DPF3a (S323) peptides. **(E)** 293T cells were transfected with myc-DPF3a and myc-DPF3a S323A. Western blotting analysis using phospho-antibody or pan-antibody of DPF3a shows that homemade phospho-DPF3a antibody fails to recognize the phospho-dead S323A mutant.

Supplementary Figure S3



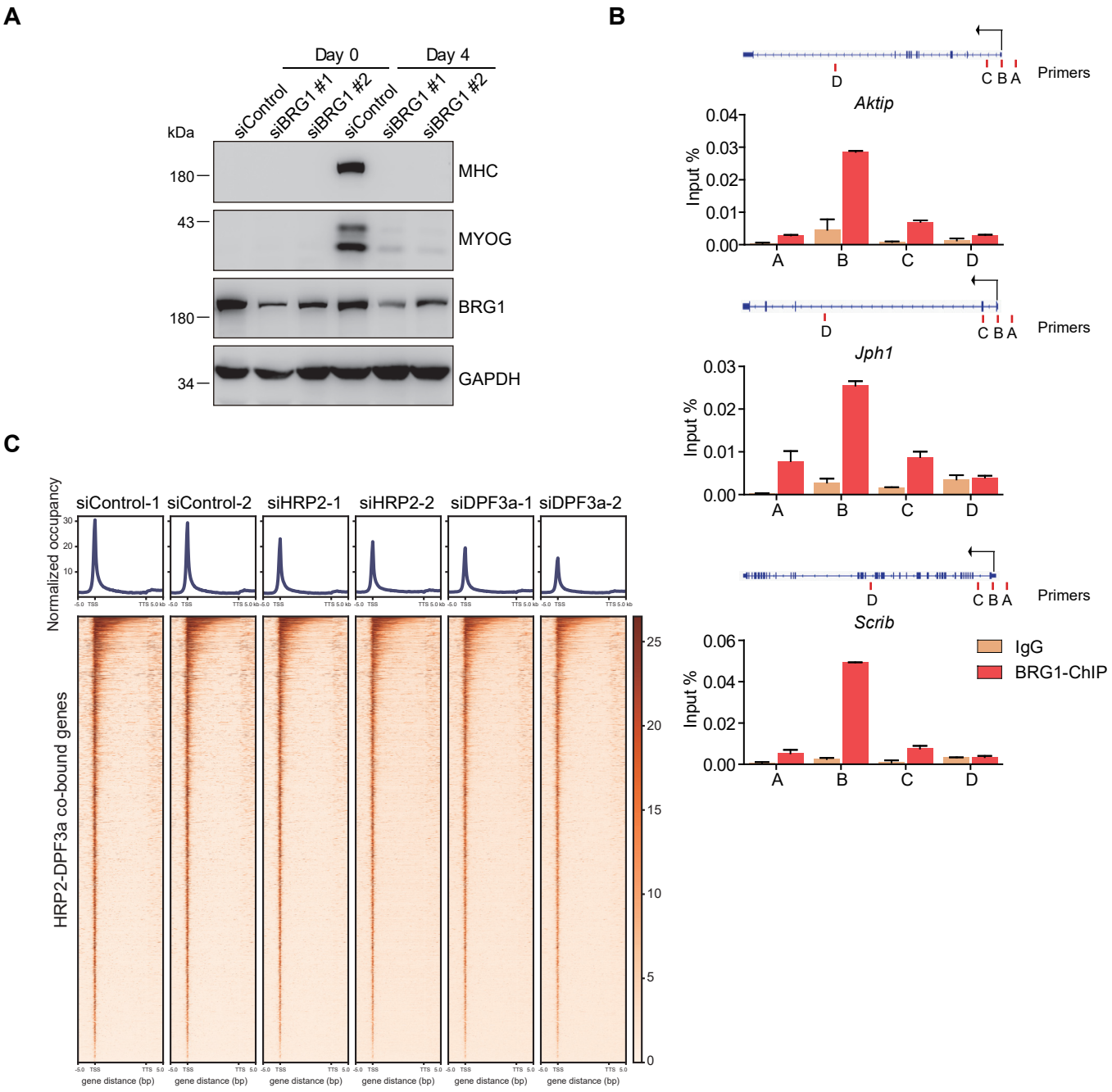
Supplementary Figure S3. HRP2 genome recruitment depends on the H3K36me2 mark. (A) Western blotting analysis of TY1, HRP2 and GAPDH in WT, TY1-HRP2 and TY1-HRP2 W21A stable cells as indicated. Expression level of endogenous HRP2 and TY1-HRP2 are comparable. **(B)** Scatter plots showing the correlation of ChIP-seq datasets obtained using endogenous HRP2 and TY1 antibody in myoblast and myotube. Correlations were calculated by the Spearman correlation coefficient. **(C)** Ethidium bromide staining of DNA after phenol-chloroform extraction from the purified mononucleosomes from 293T cells transfected with empty vector or FLAG-tagged HRP2 (WT or W21A mutant). **(D)** 293T cells expressing FLAG-tagged HRP2 (WT or W21A mutant) were subjected to protein-protein ChIP assay. Chromatin pulled down with anti-FLAG antibody was analyzed by western blotting using antibodies against various histone marks. **(E)** One quarter of HRP2-H3K36me2-co-bound genes with highest H3K36me2 and HRP2 signals as shown in Figure 6D was chose to plot profile around TSS region. Average genome-wide H3K36me2 (upper), HRP2 (middle) and both of their (lower) occupancies are shown for this gene sets. Sequences 3 kb upstream and downstream of the TSS are also included. **(F)** Western blotting analysis of NSD2, HRP2 and GAPDH in myotube transfected with scramble (siControl) and two different NSD2 siRNAs as indicated.

Supplementary Figure S4



Supplementary Figure S4. Genome-wide colocalization of DPF3a and HRP2 in myotubes. Violin plots showing the distribution of expression levels **(A)** and gene length **(B)** of HRP2-DPF3a co-bound genes and DPF3a unique genes. **(C)** Muscle-specific genes were downloaded from Housekeeping Transcript Atlas database (http://www.housekeeping.unicamp.br/). Total of 878 genes were collected as muscle-specific genes. By intersecting the muscle-specific genes with HRP2-DPF3a co-bound/DPF3a unique gene sets, 7.56% and 0.48% genes were obtained, respectively. **(D)** Heatmaps showing the distribution of DPF3a and HRP2 on DPF3a bound genes for the region -5 kb from the TSS to +5 kb from the TTS. Genes on the heatmaps are ordered according to decreasing total level of DPF3a (upper). Average genome-wide DPF3a and HRP2 occupancies are shown for DPF3a bound genes. Sequences 5 kb upstream of the TSS and 5 kb downstream of the TTS are also included (down). **(E)** ChIP-qPCR of HRP2, DPF3a and H3K36me3 at *Aktip*, *Slc12a2* and *Scrib* gene loci in MT C2C12 cells transfected with siRNA as indicated. PCR primers were designed as indicated in Figure 6G. Enrichment values (Y axis) are expressed as fold change normalized to input chromatin. Data are represented as means ± SD of three replicates. ** $P < 0.01$ and *** $P < 0.001$.

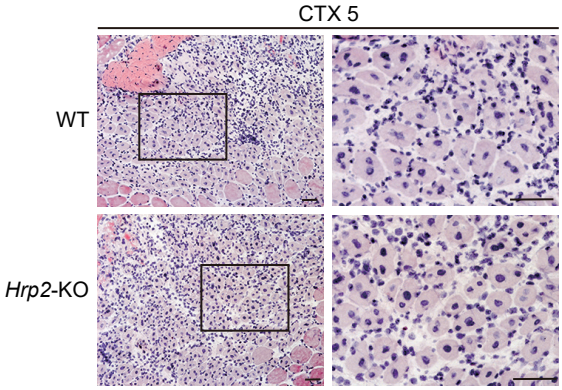
Supplementary Figure S5



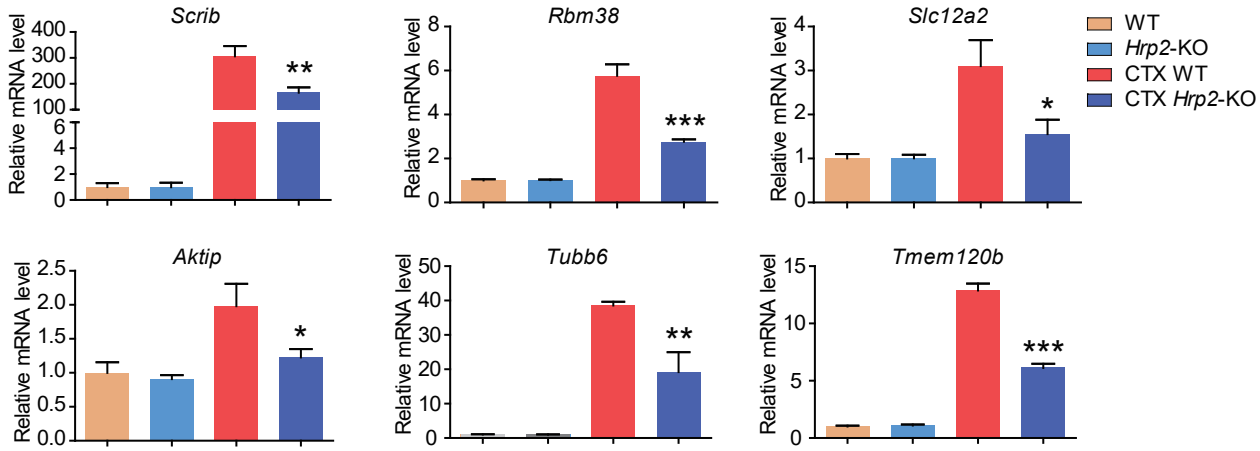
Supplementary Figure S5. HRP2 and DPF3a recruit BRG1 to target gene loci. (A) Western blotting analysis of MYOG, MHC, BRG1 and GAPDH in myoblasts and myotubes transfected with scramble and two different BRG1 siRNAs as indicated. **(B)** ChIP-qPCR of BRG1 at *Aktip*, *Jph1* and *Scrib* gene loci in MT C2C12 cells. PCR primers were designed as indicated in Figure 6G. Enrichment values (Y axis) are expressed as fold change normalized to input chromatin. Data are represented as means ± SD of three replicates. ** $P < 0.01$ and *** $P < 0.001$. **(C)** Heatmaps showing the genome-wide ATAC signal of C2C12 myotube transfected with siRNA as indicated on HRP2-DPF3a co-bound genes for the region -5 kb from the TSS to +5 kb from the TTS. Genes on the heatmaps are ordered according to decreasing total level of ATAC signal.

Supplementary Figure S6

A



B



Supplementary Figure S6. Muscle regeneration is impaired in *Hrp2* KO mice treated with CTX. (A) Representative H&E-stained cross-sections of TA muscles from male WT and *Hrp2*−/− mice. Sections were obtained from CTX-injured muscles at Day 5 post-treatment. Boxed areas are enlarged in the panels to their right. Scale bar, 50 μm. **(B)** mRNA levels of 6 representive HRP2 and DPF3a co-regulated genes were analyzed in TA muscle of WT or *Hrp2*−/− mice at Day 3 post-CTX-induced injury (n = 3 per group).

Supplementary Table S1. Primers for qPCR

Name	Forward primer	Reverse primer
mus-Scrib	CTGAGCCGTGTCAGTGTCAT	ACTCAGCCTCTTCTCCTCCT
mus-Rbm38	GTGACCATGGCAGATCGGG	TAGTGAGGAGTCAGCCCGTA
mus-Slc12a2	TGGAGTTGTGGTTATCCGCC	TGCCATCCTCTTCCTCATCTTT
mus-Aktip	GACAGCCGGAGATGCTGTT	AGCATTCACACTGCCTGGAT
mus-Tubb6	AGAAGTACGTACCCAGGGCT	CCCGTCTGTCCGAAGATGAA
mus-Tmem120b	AGGAGCTGCAGGAGACACAC	GGAATGGCGCTTGTACCTCT
mus-Dpf3a	CAGACGGGACAGTCATTCCTAAT	CTCCCAAATGAGCAGAGCGT
mus-Gapdh	TCAAGCTCATTTCCTGGTATGACA	TAGGGCCTCTCTTGCTCAGT
mus-Hrp2	CTTTGGAACGCATGAAACGG	TGTTGGGCTTTCCGTACTTG
mus-MyoD	CGCCACTCCGGGACATAG	GAAGTCGTCTGCTGTCTCAAAGG
mus-Myog	AGCGCAGGCTCAAGAAAGTGAATG	CTGTAGGCGCTCAATGTACTGGAT
mus-eMyHC	TCCAAACCGTCTCTGCACTGTT	AGCGTACAAAGTGTGGGTGTGT

Supplementary Table S2. Primers for ChIP-qPCR

Name	Forward primer	Reverse primer
Usp38-A	ACTCCAATGCCTTAAATGGCTGG	GAGCCACCAGTACTCTCTCTAC
Usp38-B	GAACACCCTGAACCCGGAAG	GGAAATGGGTCGAACGTAGC
Usp38-C	CCTGGGAGACATACTCCCGT	GCGACCTCCTGACCGATTTC
Usp38-D	AAGGAGTTTCAGTCATTGTTGCATT	AAGGCTTGAGCCATGGTACTC
Aktip-A	TGTCATTCAAGAGGCAGGGC	GTTCTCAAGCCCCTGACAGA
Aktip-B	CTCTCTCGCCGTCCATCAAA	CGAGCGGCGCTAACTTGATT
Aktip-C	CCGGGGCACAATCCCATTTC	CAGCCTTACATCACCTCGGG
Aktip-D	AGTGGAGCAATAGCACGAGG	CTGGTCCTAAGCCACGACAC
Slc12a2-A	AAGTTCCAGTCCAAACGCCC	GCATCACTGAGGTGACCGTT
Slc12a2-B	CTAAGGGAAACCGCCCACAG	CTCTGGACTCCTCTCGCTTC
Slc12a2-C	AACACTGGGGGTTAAAGGCG	TCCCAGCAAGAGCAACAAGT
Slc12a2-D	AGATGAGGAAGAGGATGGCAAG	AAATGATGAACAATGCCTGCCT
Scrib-A	AGGGGATGGTGACCGGAATA	GGGTCTGTCTTCAGCTTCCC
Scrib-B	CGTTCAATACGAGCTGTAGGCT	TAAGAAAGCGCTGCCGGG
Scrib-C	GCTGAGGGGGACTCTCCTAA	TGGGAGCTGCTGATGTCTTG
Scrib-D	ACCTCACAGCTCACAGAGGA	CTCCATGACCCCCAACTCAC
Jph1-A	GGCCACTGGGAAAATGAGGA	CGGACCAAACCCTCAGAACA
Jph1-B	GCAACACCTACCAGGGCTAC	CATCCACTTGCCCTTCGTCT
Jph1-C	TACTCCAGGTCCCCTCGTAG	AAGGGCAAGTGGATGTACCG
Jph1-D	TCTGGGGTAAATATTCTTGATGGT	TTCTTAAGCTACTTTTCTTCTGAGC