SUPPLEMENTARY INFORMATION

RIF1 regulates early replication timing in murine B cells

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Supplementary Figure 1
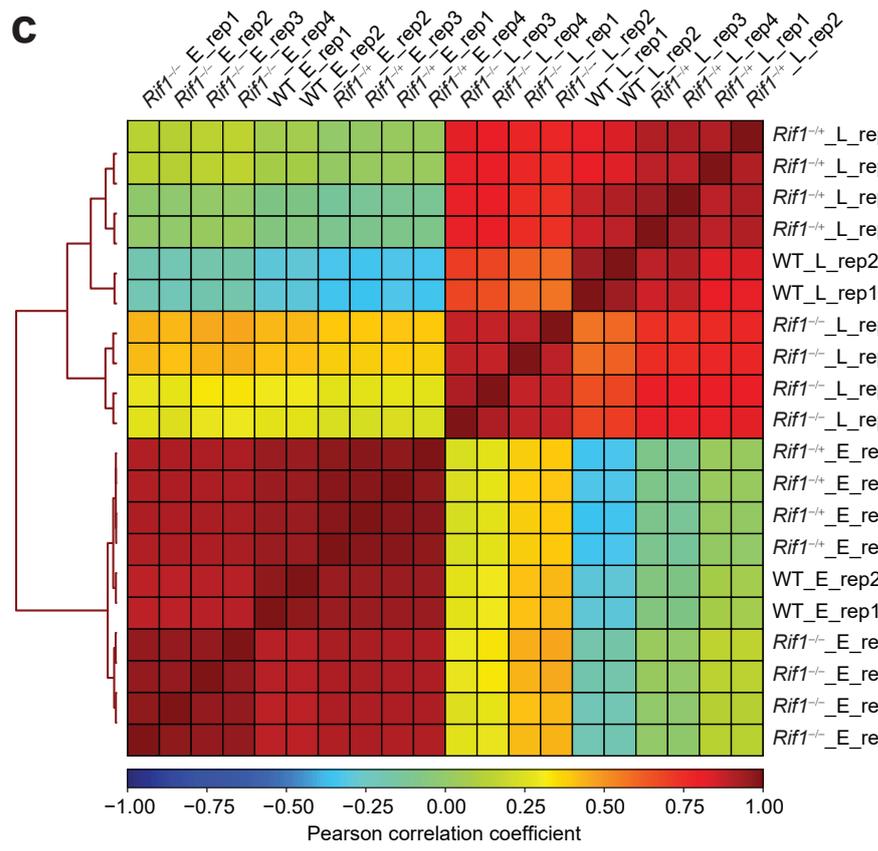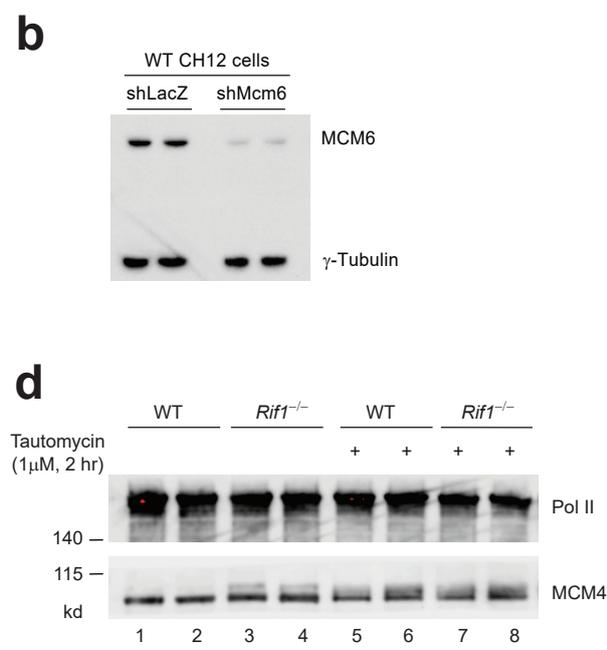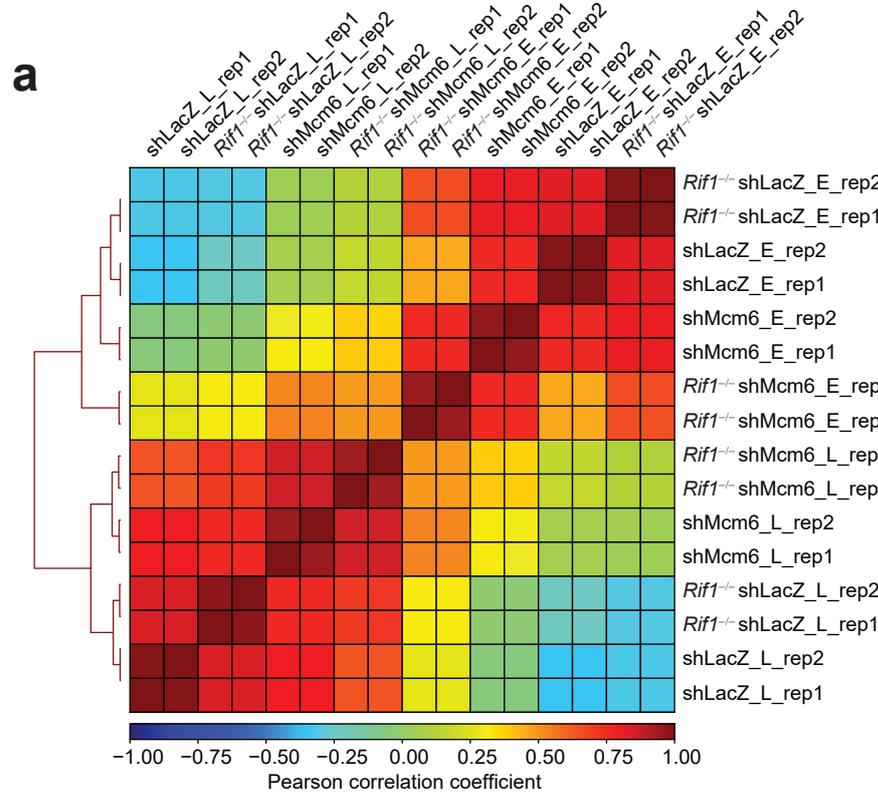
Supplementary Fig. 1 | Analysis of Repli-seq replicates. a, Pearson correlation matrix of Repli-seq data from two replicates (rep1 and rep2) of shLacZ, shMcm6, *Rif1⁻/⁻*shLacZ and *Rif1⁻/⁻* shMcm6 CH12 cells. E and L indicate the early and late fractions, respectively. The color gradient (bottom bar) corresponds to the Pearson correlation coefficient. **b,** Western blot for MCM6 protein in nuclear extracts from shLacZ and shMcm6 cells. The experiment was performed twice and results from both experiments are shown with γ-Tubulin serving as the loading control. In all cases, 20 μg of extract was loaded. Source data are provided as a Source Data file. **c,** As in **a** from WT (two replicates), *Rif1⁻/⁺* (four replicates) and *Rif1⁻/⁻* (four replicates) primary, activated splenic B cells. **d,** MCM4 phosphorylation in chromatin fractions of WT and *Rif1⁻/⁻* CH12 cells ± 1μM tautomycin for 2 hr. The experiment was performed twice and results from both experiments are shown. Pol II is used as loading control. All lanes contain 10 μg protein. Source data are provided as a Source Data file.

Supplementary Figure 2

a

n = 8,639 (34 up, 117 down)

Log2 Fold-change (*Rif1⁻ᐟ⁻* clone 1/WT)
Expression in WT (log10 TPM)

b

n = 8,639 (15 up, 181 down)

Log2 Fold-change (*Rif1⁻ᐟ⁻* clone 2/WT)
Expression in WT (log10 TPM)

c

-log10 (*P* value)

GO:0007611: Learning or memory (*Adrb2, Map1a, Ptgs1, Plk2*)
R-MMU-3247509: Chromatin modifying enzymes (*Padi3, Smarcc2, Arid5b*)
GO:0003018: Vascular process in circulatory system (*Adrb2, Ptgs1, Pde2a*)
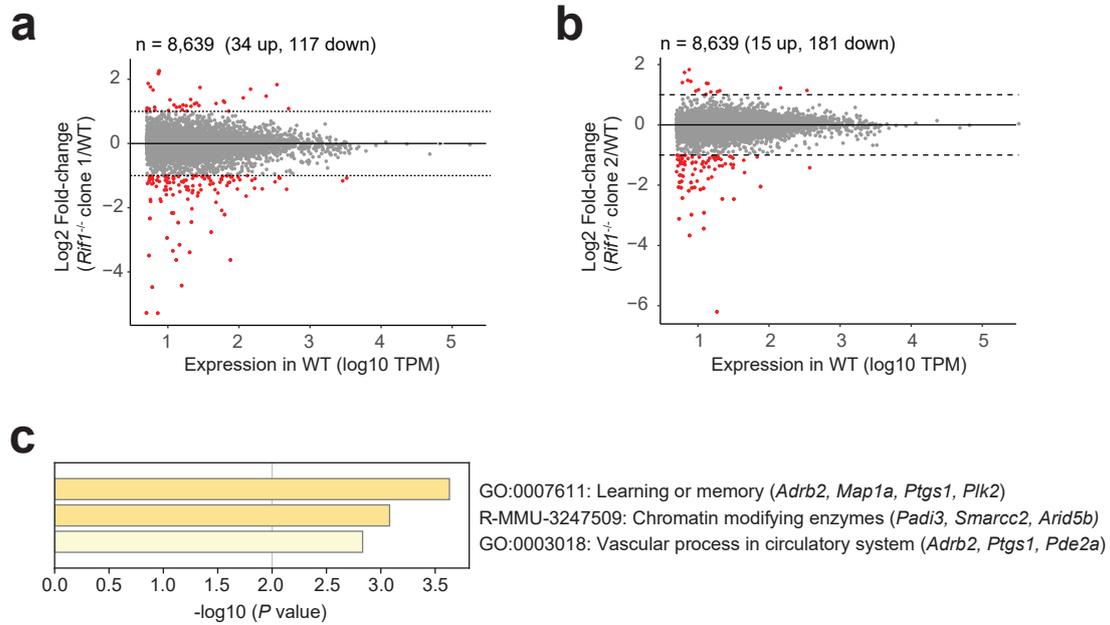
Supplementary Fig. 2 | RNA-seq analysis from *Rif1⁻ᐟ⁻* CH12 cells. a, RNA-seq analysis of *Rif1⁻ᐟ⁻* clone 1 compared to WT cells. The data are shown as a scatter plot of RNA-seq read densities in transcripts per million (TPM) versus fold-change (FC; *Rif1⁻ᐟ⁻*/WT) for 8,639 expressed genes (defined as TPM > 5 in WT cells). The dotted lines mark log2 FC 1 or -1 corresponding to 2-fold upregulated and 2-fold downregulated genes, respectively. See also Supplementary Data 1. **b,** As in **a** but for *Rif1⁻ᐟ⁻* clone 2 compared to WT cells. **c,** GO term analysis of the downregulated genes common to *Rif1⁻ᐟ⁻* clones 1 and 2 (n = 22). There were no enrichments for the common upregulated genes (n = 3). See also Supplementary Data 1.

Supplementary Figure 3

a

b RIF1 occupancy in primary B cells

c RIF1 occupancy in MEFs

d Peaks RADs

e Primary B cells MEFs
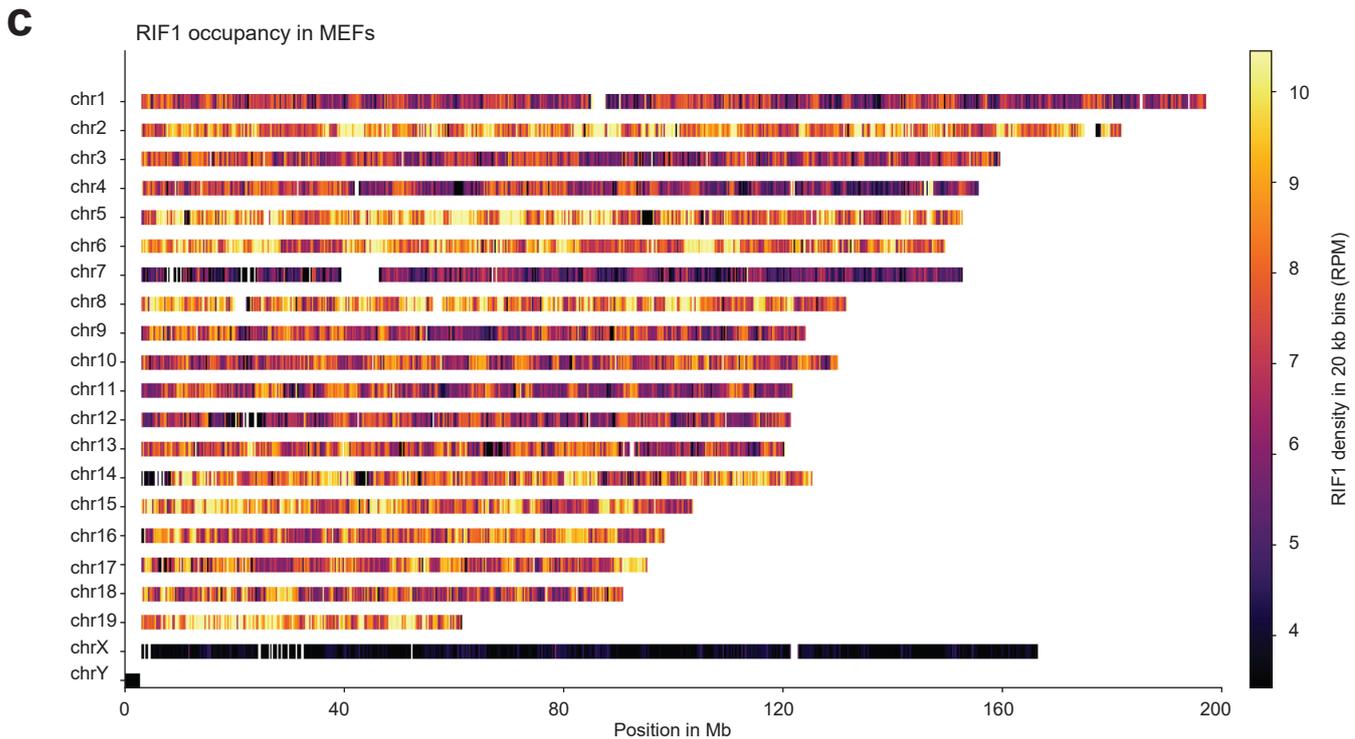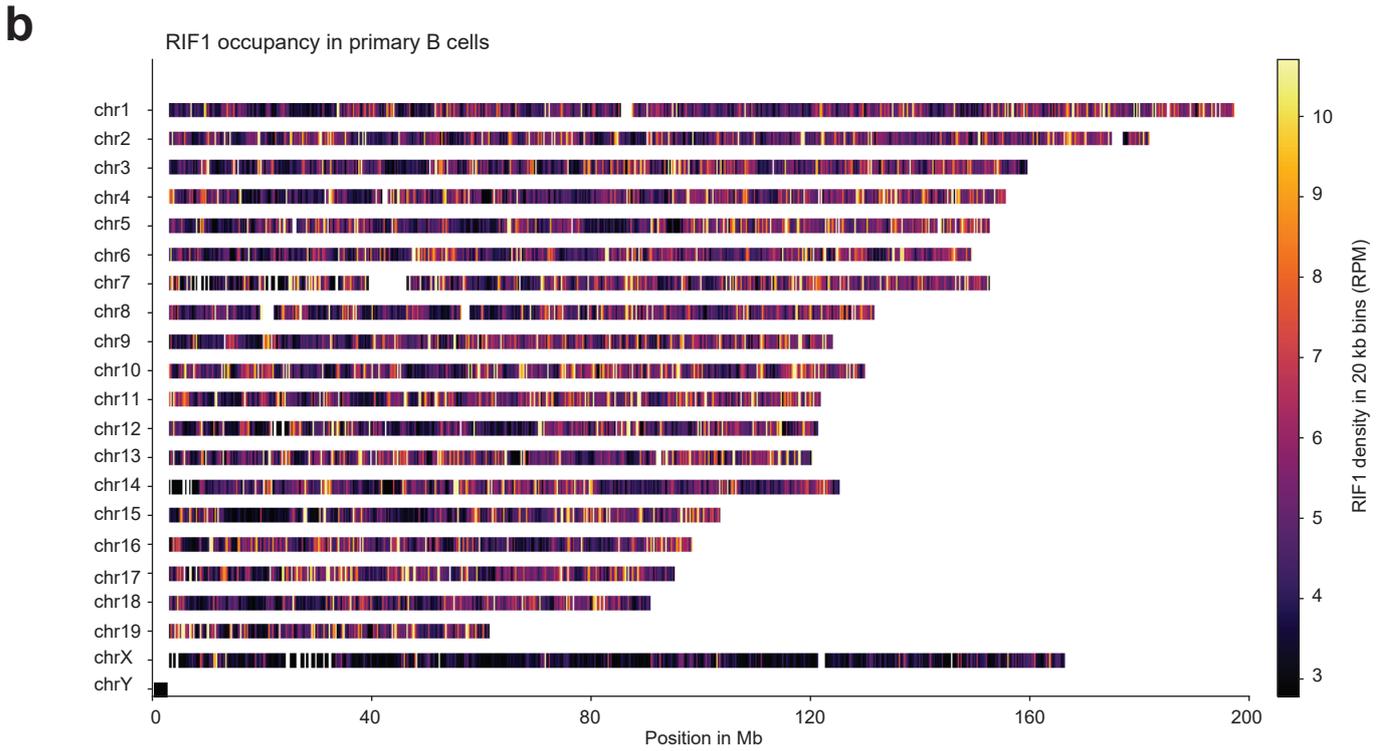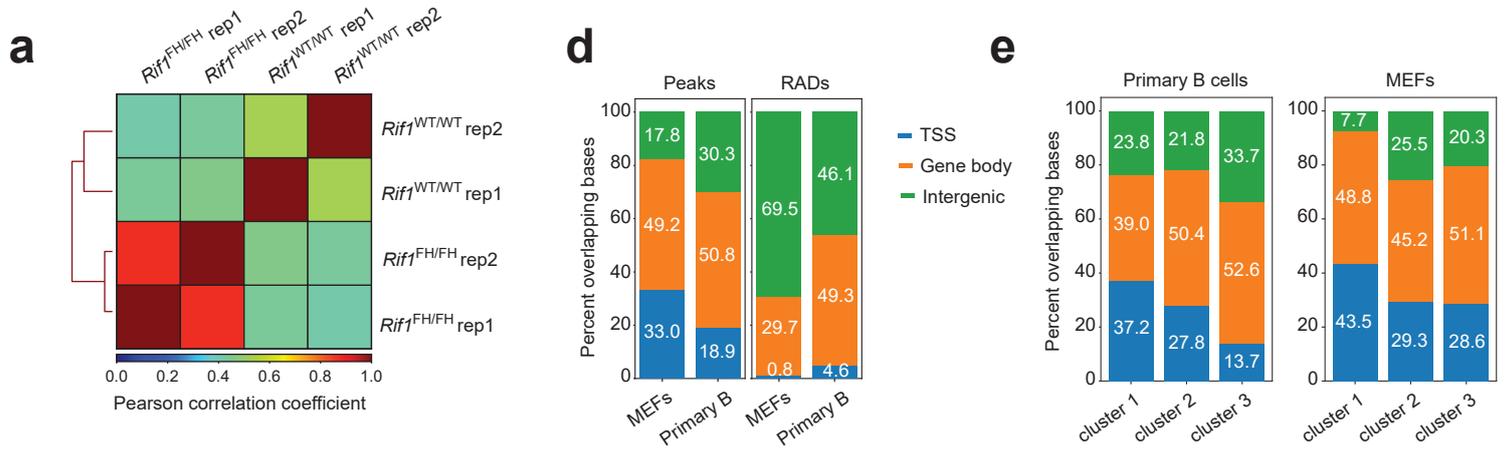
Supplementary Fig. 3 | Analysis of RIF1 ChIP-seq from primary B cells and MEFs. a, Pearson correlation analysis of RIF1 ChIP-seq datasets. Two replicates from *Rif1*^{FH/FH} and *Rif1*^{WT/WT} primary B cells were analyzed. **b,** RIF1 density from ChIP-seq in primary B cells shown across all mouse chromosomes in 20 kb genomic bins. **c,** As in **b** but for RIF1 ChIP-seq data from MEFs. **d,** Distribution of RIF1 peaks and RADs at TSSs (blue), gene bodies (orange) and intergenic regions (green). **e,** Distribution of RIF1 peaks from primary B cells (left) and MEFs (right) in TSSs (blue), gene bodies (orange) and intergenic regions (green) following k-means clustering described in Fig. 3e-f.

Supplementary Figure 4
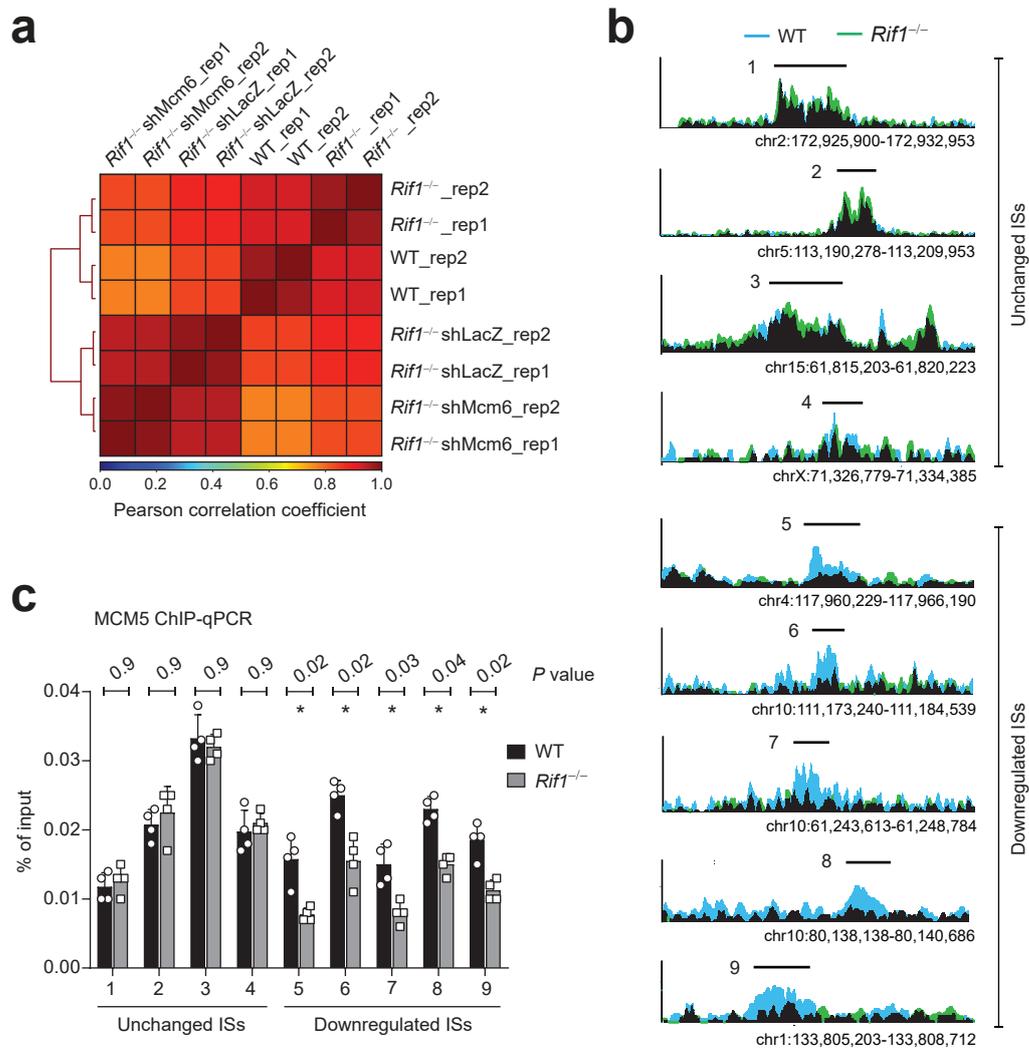
Supplementary Fig. 4: Correlation between SNS-seq replicates and analysis of RIF1 in origin licensing. a, Pearson correlation analysis of SNS-seq data from CH12 cells. Two replicates from each indicated condition were analyzed. **b,** Genomic snapshots of SNS-seq showing ISs that are unchanged (top four) or downregulated (bottom five) in *Rif1*⁻/⁻ relative to WT CH12 cells. The tracks are overlaid with WT in blue, *Rif1*⁻/⁻ in green and black being the overlap between them. The black bars indicate the ISs probed in **c** below. **c,** MCM5 ChIP-qPCR at the ISs highlighted in **b**. Data are from four experiments (N = 4) and represented as the mean ± standard deviation. Each sample is shown as a circle. Asterisks denote *P* < 0.05 via the unpaired Student's t test. Actual *P* value are shown at the top. Source data are provided as a Source Data file.

Supplemental Figure 5
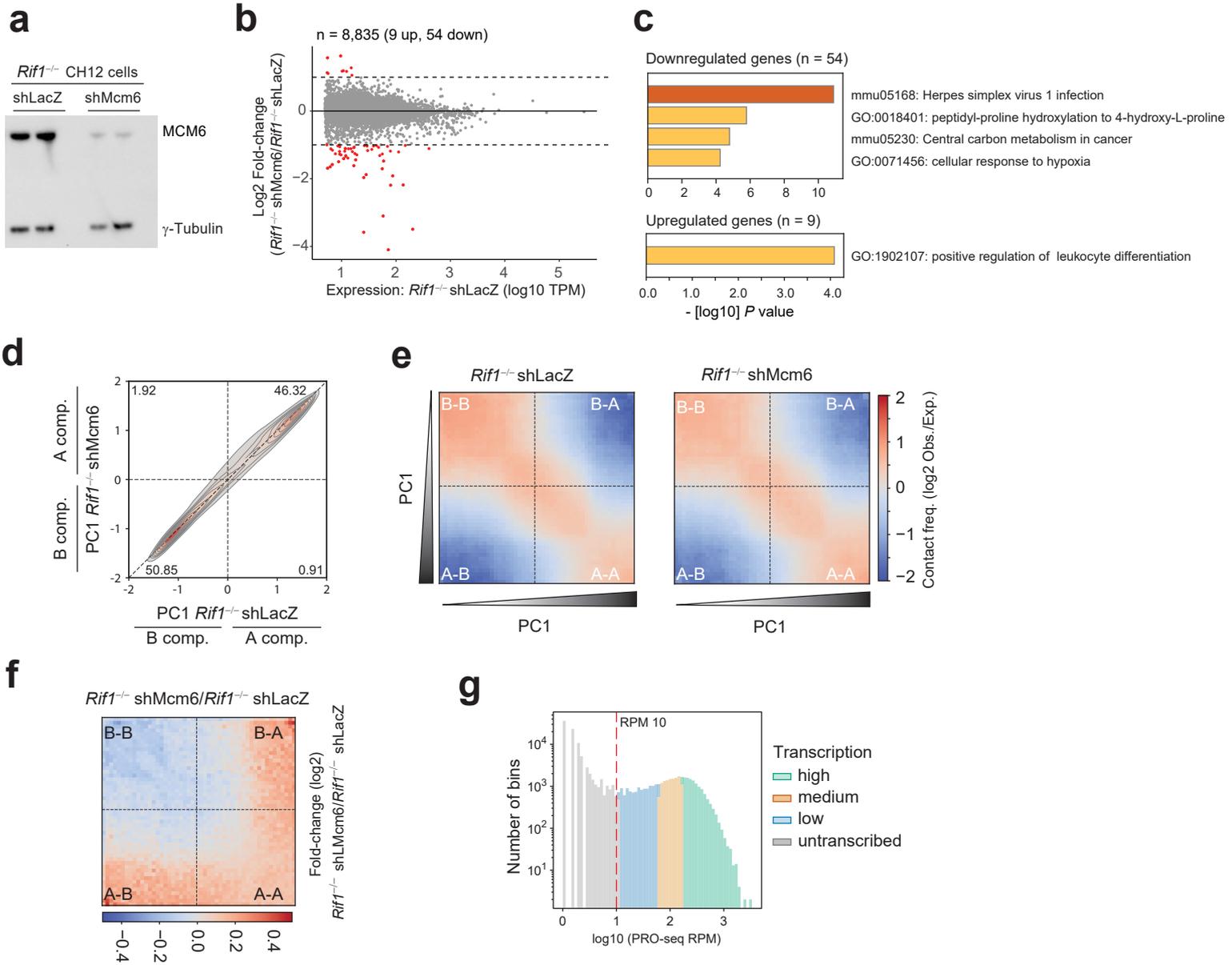
Supplemental Fig. 5 | Gene expression and genome compartmentalization analyses in *Rif1*^{−/−} shMcm6 CH12 cells.

a, Western blot for MCM6 protein levels from nuclear extracts of *Rif1*^{−/−} shLacZ and *Rif1*^{−/−} shMcm6 CH12 cells. The experiment was performed twice and results of both are shown. In all cases, 20 μg of extract was loaded. γ-Tubulin serves as the loading control. Source data are provided as a Source Data file. **b,** Scatter plot of RNA-seq read densities in TPM versus fold-change (*Rif1*^{−/−} shMcm6/Rif1*Rif1*^{−/−} shLacZ) at 8,835 expressed genes (defined as TPM > 5 in *Rif1*^{−/−} shLacZ cells). The dashed lines mark log2 FC 1 and -1 corresponding to 2-fold upregulated and 2-fold downregulated genes, respectively. **c,** GO term analysis of the 54 downregulated and 9 upregulated genes from **b**. **d,** Density-contour plot comparing PC1 compartment signals in 20 kb genomic bins from *Rif1*^{−/−} shLacZ and *Rif1*^{−/−} shMcm6 cells. **e,** Saddle plots comparing compartmental interactions between *Rif1*^{−/−} shLacZ and *Rif1*^{−/−} shMcm6 cells. **f,** Fold-change saddle plots based on data from **e**. **g,** Histogram showing the distribution of PRO-seq densities (RPM) in 20 kb genomic bins from shLacZ CH12 cells. High, Medium, Low and Untranscribed groups are color-coded to match the analysis in Fig. 7b. The dashed red line indicates RPM 10 which marks the boundary between transcribed and untranscribed regions in our analyses.